EFFECTS OF PRODUCT STRUCTURE, TEMPERATURE, WATER ACTIVITY, AND STORAGE ON THE THERMAL RESISTANCE OF *SALMONELLA* ENTERITIDIS PT 30 IN LOW-MOISTURE FOODS

By

Pichamon Limcharoenchat

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ABSTRACT

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The elevated and dynamic thermal resistance of *Salmonella* on/in low-moisture foods is an emerging challenge for the food industry. Therefore, the overall goal of this study was to improve the validation process for low-moisture foods by providing new knowledge about the effects that product structure and water activity have on *Salmonella* thermal resistance in or on low-moisture foods. The specific research objectives were: (1) To quantify the effect of inoculation protocol on the thermal resistance of *Salmonella* Enteritidis PT 30 in fabricated low-moisture foods (almond, wheat, and date products), (2) To evaluate the effects of long-term storage on the survival and thermal resistance of *Salmonella* Enteritidis PT 30 on almonds, and (3) To develop *Salmonella* thermal inactivation models that account for the effects of product structure, temperature, and water activity (for almond, date, and wheat products).

For pre- and post-fabrication protocols, samples were inoculated before and after product fabrication. *Salmonella* exhibited greater thermal resistance on almond and date products (almond meal, almond butter, and date paste) inoculated using the pre-fabrication method as compared to the post-fabrication method. However, the opposite was true for wheat products (meal and flour). Differences in the food product composition may have contributed to these findings. Based on these results, the pre-fabrication method was chosen for all further experiments in this dissertation.

In the long-term storage study, *Salmonella* populations decreased by ~3 log CFU/g after 103 weeks of storage. However, *Salmonella* thermal resistance did not significantly change during long-term storage.

Primary (log-linear and Weibull) and secondary (Bigelow-type) inactivation models for *Salmonella* were fit to isothermal inactivation data from eight different products, accounting for product structure (kernels/pieces/meal/flour/butter/paste), temperature (70-90°C), and water activity (0.25-0.65 a_w). Overall the log-linear model was the most-likely-correct model, and the Bigelow-type secondary models therefore were incorporated into the log-linear model.

Among all products, *Salmonella* was most heat resistant in 0.25 a_w almond meal ($D_{80°C}$ = 75.2 min), and least resistant in 0.65 a_w date paste ($D_{80°C}$ = 0.7 min). Decreasing a_w increased thermal resistance. Additionally, *Salmonella* thermal resistance was generally greater on fabricated than whole products. However, these differences were relatively small for wheat products. *Salmonella* resistance on fabricated wheat products actually was lower than on wheat kernels at 0.45 and 0.65 a_w. Variability in some of these effects across products might be attributable to compositional factors (e.g., sugar or moisture content), temperature-induced shifts in sorption isotherms or physical properties, or variable effects of particle sizes and microenvironment within the fabricated products.

Overall, the primary-secondary inactivation models fit the various data sets well (RMSE from 0.51 to 1.08 log) and therefore are potential tools to predict *Salmonella* thermal inactivation for these products. Ultimately, this dissertation shows that low-moisture process validation protocols should account for inoculation methods and specific product structures, both of which can significantly affect process outcomes.

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1 INTRODUCTION

1.1 Problem Statement

Outbreaks of salmonellosis and recalls associated with low-moisture foods have increased in recent years. From 1996 to 2009, *Salmonella* cases increased by more than 20% in the United States, and the ability of outbreak detection by the PulseNet system (Pulsed-field gel electrophoresis: PFGE) increased illnesses reported by almost 10% (Scharff et al., 2016). The PulseNet system has helped improve the detection of outbreaks, but at the same time recalls have also increased during this period. In 2015, the U.S. Food and Drug Administration (FDA) reported that over 30 *Salmonella*-linked recalls were attributed to low-moisture food products (U.S. Food and Drug Administration, 2016a). Additionally, low-moisture food products, such as poppy seeds (U.S. Food and Drug Administration, 2016b) and ginger powder (U.S. Food and Drug Administration, 2017), were recalled in 2016 and 2017 due to *Salmonella* contamination. The Center for Disease Control and Prevention (CDC) reported multistate outbreaks of *Salmonella* in sprouted nut butter spreads (Centers for Disease Control and Prevention, 2016a) and pistachios (Centers for Disease Control and Prevention, 2016b) in 2016. Two people from the pistachio outbreaks were hospitalized.

Outbreaks of salmonellosis linked to low-moisture foods, including almonds, have occurred throughout the world (Isaacs et al., 2005). Peanut butter (Centers for Disease Control and Prevention, 2007), wheat flour (McCallum et al., 2013), unsweetened cereal (Russo et al., 2013), and chocolate (Werber et al., 2005) are all additional examples. Further, 75 people in New Zealand were infected by consuming contaminated raw flour from an uncooked baking mixture from October 2008 to January 2009 (McCallum et al., 2013). Moreover, a variety of low-moisture foods, such as bleached flour, raw macadamia nuts, pistachios, almond butter, and ginger powder were

recalled due to contamination from *Salmonella* (U.S. Food and Drug Administration, 2014b, 2015, 2016; U.S. Food and Drug Administration, 2015, 2017).

Compared to other pathogenic organisms in food products, *Salmonella* in low-moisture foods is highly resistant to lethal treatments and able to survive long periods (Blessington et al., 2012; Kimber et al., 2012). Also, this pathogen can remain viable in water for up to a week and in soil for over a year (Adams and Moss, 2008). For example, *Salmonella* Montevideo survived on red winter wheat during 28 weeks of storage at a relative humidity of 13% (Crumrine and Foltz, 1969). In a date paste, *Salmonella* decreased during storage, but was still detected after 8 months at 4°C (Beuchat and Mann, 2014).

Standard hygiene and sanitation practices are designed to prevent and control *Salmonella* from contaminating incoming raw materials and ingredients. Environmental monitoring and control also are important steps to minimize pathogens in food products (Chen et al., 2009b). For example, environmental contamination and substandard sanitation were the likely origin of a *Salmonella* outbreak traced to a peanut butter factory (Sheth et al., 2011; Viazis et al., 2015). In the case of cereal products linked to *Salmonella* Agona outbreaks, the pathogen was detected in environmental samples within the production facility (Russo et al., 2013).

Outbreaks of salmonellosis not only affect people's health, but also affect the economy via loss of product in the market, recall costs to the manufacturer, lost productivity, and a decrease in sales. The U.S. Department of Agriculture's Economic Research Service (USDA-ERS) estimated the medical costs due to salmonellosis at $3.7 billion per year (U.S. Department of Agriculture, 2014). *Salmonella* was the leading cause of medical costs from foodborne outbreaks in the United States in 2015 (News desk, 2015), with an estimated cost of illness for *Salmonella* infection at $1,792 per case (Scharff et al., 2016). Based on one estimate, Weise (2009) reported that the 2009

outbreak/recall due to *Salmonella* in peanut butter cost $1 billion, with the Kellogg Company alone estimated to have lost $70 million in that recall, which was one of the largest food recalls in the history of the United States. Such recalls can also negatively affect customer confidence in food safety and therefore reduce sales of the products affected.

Food product characteristics, such as water content and water activity, are important factors that impact *Salmonella* survival [See Chapter 2 for detailed in discussion]. *Salmonella* thermal resistance in low-moisture food products, such as wheat flour and peanut paste, increases as water activity decreases (Kataoka, 2014; Smith and Marks, 2015). He et al. (2013) reported that *Salmonella* thermal resistance in peanut butter at 90°C was significantly reduced when the water activity increased. For different food products at the same water activity levels, thermal resistance also can be affected by chemical composition and the type of product. For example, *Salmonella* thermal resistance in all-purpose flour is significantly lower than in peanut butter at 0.45 a_w (Syamaladevi et al., 2016a); however, slight changes of fat content in different peanut butter products did not affect the heat resistance of *Salmonella* (Kataoka, 2014).

Limited studies have shown that other factors can affect *Salmonella* thermal resistance in low-moisture foods, such as sodium chloride concentration, type of sugar, and fat content (D'Souza et al., 2012; Kataoka, 2014; Mattick et al., 2001; Shrestha and Nummer, 2016). However, no known prior studies have evaluated the effect of varying physical structures of low-moisture foods on *Salmonella* thermal resistance. In contrast, there have been several such studies with high-moisture foods (Mogollon et al., 2009; Tuntivanich et al., 2008; Velasquez et al., 2010), which reported that physical structure did impact *Salmonella* thermal resistance in raw pork, beef, and turkey. Thermal resistance of *Salmonella* in whole-muscle beef and turkey was 50% greater than in ground products of equivalent composition at 55, 60, and 62.5°C (Mogollon et al., 2009;

Tuntivanich et al., 2008). Similarly, *Salmonella* thermal resistance in ground pork was 0.64 to 2.96 times lower than in whole muscle pork cooked at 55 to 63°C (Velasquez et al., 2010). Although these were all high-moisture food systems, the results do suggest that product structure (given equivalent chemical composition, moisture content, and temperature) might also affect *Salmonella* thermal resistance in other food materials, such as low-moisture foods.

Currently, there is no known prior research regarding the effects of product structure (in combination with water activity and temperature) on *Salmonella* thermal resistance in low-moisture food products. Specifically, if product structure affects the thermal response of *Salmonella* in low-moisture foods, then this could have a significant impact on food safety, especially because product structure has not typically been considered as a contributing factor in inactivation models or process validations.

1.2 Research Goal, Objectives, and Hypotheses

The overall goal was to improve pasteurization validations for low-moisture foods by providing new knowledge about the effects of product structure and water activity on *Salmonella* thermal resistance in or on low-moisture foods (almond, date, and wheat products).

The specific research objectives were:

1. To quantify the effect of inoculation protocol on the thermal resistance of *Salmonella* Enteritidis PT 30 in fabricated low-moisture foods (almond, wheat, and date products).
2. To evaluate the effects of long-term storage on the survival and thermal resistance of *Salmonella* Enteritidis PT 30 on almonds.

3. To develop *Salmonella* thermal inactivation models that account for the effects of product structure, temperature, and water activity (for almond, date, and wheat products).

The hypotheses of this research were that: (1) Inoculation protocols impact *Salmonella* thermal resistance on or in almond, date, and wheat products, (2) Thermal resistance of *Salmonella* on almond kernels does not change during long-term storage (up to 2 years), (3) *Salmonella* thermal resistance on or in almond, date, and wheat products increases with decreasing water activity, regardless of product structure, and (4) Product structure of almond, date, and wheat products significantly affects the *Salmonella* thermal resistance.

2 LITERATURE REVIEW

Low-moisture foods come in a variety of categories, such as nuts, fruits, and wheat products. Among various factors previously assessed, three were found to have the greatest impact on *Salmonella* thermal resistance: water activity (Syamaladevi et al., 2016b), inoculation protocol (Hildebrandt et al., 2016), and temperature (Smith et al., 2016). This review highlights previous literature that has conveyed basic information on low-moisture foods, and, more specifically, on product factors (water, physical, and chemical properties) that have the largest impact on both the survival and thermal inactivation of *Salmonella* in low-moisture systems.

2.1 Low-Moisture Foods of Interest

To date, prior studies on *Salmonella* thermal resistance in low-moisture foods have typically included only one specific food category such as peanut butter (Li et al., 2014a), almonds (Abd et al., 2012), or wheat flour (Syamaladevi et al., 2016a), but have not encompassed comparisons on the basis of physical structure. In this dissertation, low-moisture foods (almond, date, and wheat products) were chosen to represent high-fat, high-sugar, and high-starch products, respectively, in thermal inactivation studies.

2.1.1 Almonds

Almonds (*Prunus dulcis*) are an increasingly popular food in the United States. The California almond crop was valued at $5.2 billion during 2016-2017, with $4.4 billion exported in 2016 (Almond Board of California, 2017).

Almonds are harvested with mechanical tree shakers, shelled, sized, stored, and processed (Almond Board of California, 2018a). In the United States (California), almond pasteurization is required via an Agriculture Marketing Order, and the mandatory treatment criterion is a minimum

4-log reduction of *Salmonella* on almonds (Federal Register/Vol. 72, No. 61/Friday, March 30, 2007/Rules and Regulations, Pages 15021-15036) (U.S. Department of Agriculture, 2007). Pasteurization methods that have been approved by the FDA include oil roasting, dry roasting, blanching, stream processing, and propylene oxide (PPO) gas treatment (Almond Board of California, 2018b).

2.1.2 Dates

In the United States, dates (*Phoenix dactylifera*) are mainly produced in California and were valued at $67 million in 2016 (U.S. Department of Agriculture, 2017). During 2016-2017, the import value of fresh dates was $47 million and the United States also exported $52 million of fresh dates (U.S. Department of Agriculture, 2018).

Dates are harvested, cleaned, and sorted by size, skin condition, moisture content, and color (Riggs, 2015). There are no requirements for date pasteurization. Dates usually are directly transported to open-air markets after processing in the Middle East and North Africa. In addition, fumigation is used to eliminate insect pests in the industry (Chao and Krueger, 2007).

2.1.3 Wheat

Wheat (*Triticum*) is widely used in baked goods, such as cakes, flat breads, and cookies. In the United States, the U.S. produced and exported wheat to countries such as Japan, Mexico, and Nigeria (U.S. Wheat Associates, 2016). The estimate wheat export value for 2017 was $896 million (U.S. Wheat Associates, 2018).

Wheat milling is a major value-added contributor to the food industry in the United States (North American Miller's Association, 2016). Generally, the process of milling wheat includes

cleaning, separating, grinding, sieving, and bleaching. No requirements exist for wheat pasteurization. In some cases, the wheat flour will be enriched with vitamins or other nutrients to improve its nutritional quality (North American Miller's Association, 2016).

2.2 Salmonella

Salmonella spp. is a Gram-negative, facultative rod-shaped bacterium, that can cause foodborne infections (Adams and Moss, 2008). People infected with *Salmonella* may experience fever, nausea, diarrhea, vomiting, abdominal pain, and, in severe cases, even death (U.S. Food and Drug Administration, 2014a).

In this dissertation, *Salmonella* Enteritidis (SE) phage type 30 (PT 30) was used for experiments. This strain was responsible for a large outbreak of salmonellosis associated with almonds that occurred in Canada during 2000 to 2001 (Isaacs et al., 2005) and was also associated with a salmonellosis outbreak linked to raw almonds in 2004 (Centers for Disease Control and Prevention, 2004). Thermal resistance of *Salmonella* is influenced by serovar (Doyle and Mazzotta, 2000; Santillana-Farakos et al., 2014a) and therefore using a single serovar/strain with a given study simplified the analyses of key treatment effects.

2.3 *Salmonella* Survival in a Low-Moisture System

Salmonella can survive for long periods in dry locations and in low-moisture food products (Adams and Moss, 2008). The persistence of *Salmonella* in dry environments can affect *Salmonella* control strategies. Even though the number of microorganisms might decline over time, in some cases the rate of reduction depends on multiple factors, such as product formulation, storage temperature, and the cleaning process (Chen et al., 2009a, b).

Low-moisture foods, such as almonds, wheat flour, and peanut butter, do not support the growth of *Salmonella*; however, contamination of low-moisture products can occur at multiple points during pre- and post-harvest-processing (Scott et al., 2009). Contamination can also be caused by poor sanitation practices, poor equipment design, unsuitable maintenance procedures, and poor ingredient storage conditions (Scott et al., 2009). As an example, wheat grain and flour from wheat mills were monitored for yeast, mold, and pathogens in baseline testing between 2006 and 2007 in Queensland, Australia, where *Salmonella* was detected in wheat that was contaminated with soil, stone, and other environmental contaminants (Eglezos, 2010).

In survival/storage studies, *Salmonella* populations on almonds declined 1.8 log CFU/g and 2.1 log CFU/g after 24 and 48 weeks of storage (23°C), respectively (Abd et al., 2012; Uesugi et al., 2006). *Salmonella* populations on in-shell pecans also decreased by 2.49 log CFU/g after being stored at 21°C for 78 weeks (Beuchat and Mann, 2010). At 25°C, the population of *Salmonella* in hazelnuts, pecans, and pine nuts decreased by 1 log after 24, 34, and 52 weeks of storage, respectively (Santillana-Farakos et al., 2017). *Salmonella* populations in date paste declined 2.08 log CFU/g after 242 days of storage at 4°C, and by < 1 log CFU/g after 84 days of storage (25°C). In contrast, *Salmonella* populations in date paste homogenates with water actually increased by 2.74 log when stored at 25°C for 2 days (Beuchat and Mann, 2014).

Water activity (a_w) impacts the survival of *Salmonella*. In whey protein powder, *Salmonella* Montevideo and *Salmonella* Typhimurium survived better at 0.18 a_w than at 0.54 a_w after 6 months of storage (36°C) (Santillana-Farakos et al., 2014a). Increasing the water activity in nut products (hazelnuts, pecans, and pine nuts) decreased the survival of *Salmonella* after 52 weeks of storage (25°C) (Santillana-Farakos et al., 2017). Additionally, peanut paste with a low water activity (0.30 a_w) led to greater *Salmonella* survival when compared to the same product at 0.60 a_w after 12

months of storage at 20°C (Kataoka, 2014). However, a 9% difference in fat content in peanut paste samples did not affect the survival of during long-term storage (Kataoka, 2014).

The change in a_w and moisture content during storage of low-moisture foods has been reported in very few microbial studies survived. Kimber et al. (2012) reported that the a_w and moisture content (MC) of almonds in sealed plastic bags fluctuated (4-6% MC and 0.30-0.60 a_w) during 7 months of storage at -19, 4, and 24°C. The MC of peanuts and pecans also increased by 1.2% and 1.0%, respectively, when stored in sealed plastic bags at 4°C (Brar et al., 2015). When stored at ambient temperature in a sealed container for a full year, the MC of raw peanuts and pecans (Brar et al., 2015) and walnut kernels (Blessington et al., 2012) was stable in a sealed container when stored for a full year (3.8% MC for peanuts, 2.6% MC for pecans, and 3.0% MC for walnut kernels).

2.4 Factors that impact *Salmonella* Thermal Resistance in Low-Moisture Foods

Many factors affect *Salmonella* thermal resistance, including a_w (He et al., 2011; He et al., 2013), fat content (Kataoka, 2014), and salt content (Shrestha and Nummer, 2016). In this section, the selected factors in this dissertation (i.e., water activity, temperature, and inoculation method) are discussed relative to the pathogen response during processing.

2.4.1 Water activity

By definition, the a_w of a food product is the ratio between the vapor pressure of the food and vapor pressure of pure water (Barbosa-Cánovas, 2007). It can be calculated by the following equation:

$$a_w = \left(\frac{p_w}{p_w^o}\right)_T \quad (1)$$

where p_w is the equilibrium partial vapor pressure in the system, p_w^o is the partial equilibrium vapor pressure of pure liquid water, and T is the temperature at which the sample is measured.

A_w is the most important factor in controlling the growth of *Salmonella* in food products. *Salmonella* does not grow at a_w lower than 0.94 (Adams and Moss, 2008). Scott et al. (2009) reported that the controlled a_w in the industry was below 0.85 a_w; therefore, the prevention of *Salmonella* growth in low-moisture systems is typically based on controlled a_w.

A_w also impacts *Salmonella* thermal resistance in low-moisture foods (Syamaladevi et al., 2016b). When the water activity of a food matrix is reduced, *Salmonella* thermal resistance can increase greatly. Villa-Rojas et al. (2013) showed that the $D_{70°C}$ for *Salmonella* Enteritidis PT 30 on almond kernels at 0.601 a_w (15.5 min) was higher than the $D_{68°C}$ at 0.946 a_w (0.42 min). He et al. (2013) reported increased thermal resistance of *Salmonella* (Enteritidis, Typhimurium, and Tennessee) in peanut butter when the water activity decreased from 0.80 to 0.20 a_w, when heated at 90 and 126°C. *Salmonella* (Agona, Montevideo, and Typhimurium) was more thermally resistant in inoculated whey protein powder at 0.18 a_w than at 0.54 a_w when both products were vacuum sealed and heated at 70°C for 48 h (Santillana-Farakos et al., 2014a). Initial water activity (from 0.10 to 0.70 a_w) also impacted the viability of *Salmonella cerevisiae* in wheat flour and skim milk powder during hot air treatment (150 and 200°C) (Laroche et al., 2005).

The different water activity levels (0.30 and 0.60 a_w) did appear to affect *Salmonella* thermal resistance in inoculated wheat flour samples. The thermal resistance of *Salmonella* in rapidly-desiccated flour (0.60 a_w to 0.30 a_w in ≤ 4 min) and rapidly-hydrated flour (0.30 a_w to 0.60 a_w in 2.5 min) were similar when compared to the heat resistance in flour that was slowly equilibrated (4-6 days) to the same a_w value (Smith and Marks, 2015). Therefore, the speed of a_w change did not impact *Salmonella* thermal resistance.

Few studies have reported the impact that moisture content has on the heat tolerance of *Salmonella*. Beuchat and Mann (2011b) reported that *Salmonella* declined faster on pecan nutmeats when the initial MC was higher (2.8 vs. 11.2%) in hot air treatments (15 min at 90 and 120°C). Additionally, MC correlated with the inactivation kinetics of *Salmonella* during a moist-air heating process and was reported to be a better parameter in calculating process validations (Garcés-Vega, 2017).

The relationship between a_w and MC is described by moisture-sorption isotherms. Adsorption isotherms generally yield a lower moisture content than desorption isotherms at a given water activity, possibly due to the food structure, type of food, or the process temperature (Okos et al., 2007). The difference in the equilibrium moisture content between the adsorption and desorption isotherms at a given relative humidity or water activity is called "Hysteresis" (Okos et al., 2007). This hysteresis pattern occurs in low-moisture foods such as almond kernels (Pahlevanzadeh and Yazdani, 2005), wheat flour (Moreira et al., 2010), and dates (Chukwu, 2010). Therefore, the sorption stage of low-moisture foods may also need to be considered in thermal inactivation processes, since a_w may not sufficiently describe the water effect on thermal resistance (Garcés-Vega, 2017).

2.4.2 Temperature

Temperature is probably the most important parameter in thermal process validation in low-moisture foods (Chen et al., 2009b). Numerous studies have reported the effect of temperature on *Salmonella* inactivation in/on various of low-moisture products, such as *Salmonella* Typhimurium DT104 in low-a_w (high-sugar) broths (Mattick et al., 2001), *Salmonella cerevisiae* on wheat flour and skim milk powder (Laroche et al., 2005), *Salmonella* cocktails in peanut butter

(He et al., 2013; Ma et al., 2009; Shachar and Yaron, 2006), *Salmonella* cocktails on pecans (Beuchat and Mann, 2011a, b), *Salmonella* Enteritidis PT 30 on almonds (Abd et al., 2012; Harris et al., 2012), and *Salmonella* cocktails in dried fruits (Beuchat and Mann, 2014). Various approaches to modeling this affect are described below in section 2.6.

2.4.3 Inoculation method

Previous reports of bacterial survival or inactivation in low-moisture foods are based on a range of inoculation methods. Ideally, the inoculation methods should yield bacterial responses that reflect actual contamination and processing scenarios. For inoculum preparation, *Salmonella* strains have been grown in tryptic soy broth (TSB) (Danyluk et al., 2005; Ma et al., 2009; Smith and Marks, 2015) or brain heart infusion (BHI) broth (He et al., 2011; He et al., 2013). Bacteria were harvested and re-suspended in peptone water (Laroche et al., 2005), a binary water/glycerol solution (Smith and Marks, 2015), or peanut oil (for peanut butter) (He et al., 2011; He et al., 2013). The means of dispersing the inoculum in the food matrix was product dependent and included hand mixing (Syamaladevi et al., 2016a), machine stomaching (Smith and Marks, 2015), misting or transfer from sand (Beuchat and Mann, 2014), a mortar for food powder (Laroche et al., 2005), or a sterile wooden tongue depressor for nut butter products (Burnett et al., 2000; Ma et al., 2009). Initial pathogen inoculation levels in samples have been highly variable, ranging from 4.5 to 9.0 log CFU/g for peanut butter (Kataoka, 2014; Keller et al., 2012; Ma et al., 2009; Shachar and Yaron, 2006).

The impact of certain aspects of inoculation protocols on *Salmonella* thermal resistance in low-moisture foods has been reported in few studies. Ma et al. (2009) found that *Salmonella* thermal resistance in peanut butter increased with increasing incubation time during inoculum

preparation. Similarly, Keller et al. (2012) reported that *Salmonella* growth procedures, including temperature and growth media, also impacted *Salmonella* thermal resistance in peanut butter. More recently, Hildebrandt et al. (2016) used five different methods for inoculating *Salmonella* into wheat flour, with significantly different survival kinetics obtained at the same water activity (0.45 a_w) and temperature (80°C) . Additionally, a mist-inoculation procedure was shown to result in lower *Salmonella* survival than did a sand-inoculation procedure for stored dried fruit (Beuchat and Mann, 2014).

Many studies have examined thermal resistance of bacteria; however, very few have assessed the thermal resistance of the same *Salmonella* strains in the same product at the same a_w and temperature, such that variability in inoculation methods can affect results and impede cross study comparison. For example, at the same a_w (~0.40-0.45) and temperature (90°C), the thermal resistance of *Salmonella* Tennessee in peanut butter, when inoculated by adding strains directly into the matrix (He et al., 2013), was five times lower than in another study (Li et al., 2014a) where the strains were suspended in peanut oil prior to introduction into the peanut butter. Consequently, increasing evidence suggests that the inoculum preparation and methodologies are likely key factors affecting thermal resistance and therefore any process validation relying on the resulting inactivation data or parameters.

2.5 Changes in Physical and Chemical Properties during Fabrication and Heating

2.5.1 Microenvironment

Fabrication changes the product structure, potentially leading to different microenvironments. For example, fabricating almond into almond butter may form a two-phase system (oil and water) (Li et al., 2014b). *Salmonella* may survive and exhibit different thermal resistance in each phase. Shachar and Yaron (2006) reported that *Salmonella* was less thermally resistant in water than in the oil phase, probably because the high fat content protects *Salmonella* cells at high temperature.

Li et al. (2014b) reported *Salmonella* survival and thermal resistance in peanut butter and nonfat dry milk powder mixture. *Salmonella* was inoculated into peanut butter and milk powder before mixing. *Salmonella* populations in milk powder declined faster than in peanut butter (4-log reduction) after 5 weeks of storage at 25°C. *Salmonella* populations also had higher rate of reduction in milk powder as compared to peanut butter after heating at 90°C for 10 min (3- and 5- log reduction) (Li et al., 2014b), indicating that the microenvironment impacted *Salmonella* behavior. Also, the attachment and adherence of cells on/in selected low-moisture products after fabrication may have impacted pathogen behavior and thermal processing (Gurtler et al., 2014). These results indicate that the microenvironment around *Salmonella* (i.e., location, or attachment) may be one reason for different thermal resistances after product fabrication.

2.5.2 Thermal treatment

2.5.2.1 Calorimetry for heat transfer

Differential scanning calorimetry (DSC) can be used to evaluate many of the thermally induced physical changes that take place, such as fat crystallization in edible oil (Tan and Man, 2000), phase transitions of date palm (Zaitoon et al., 2016), or wheat grain cooking (Jankowski and Rha, 1986). During heating, the physical state of some products may change due to, for example, the denaturation of proteins. Amirshaghaghi et al. (2017) reported an irreversible denaturation of almond proteins after heating above 80°C. According to Jankowski and Rha (1986), dry grain and starch showed similar biphasic thermal transitions characterized by peaks at 64.5°C and 86°C. DSC also was used to determine phase transitions of dates for improving storage conditions to extend shelf-life (Zaitoon et al., 2016). Glass transition temperature decreased as the moisture content of dates increased. Physical changes during thermal treatment may also have impact *Salmonella* thermal resistance.

2.5.2.2 Water activity at high temperatures

Water activity plays an important role in the heating process. Syamaladevi et al. (2016a) reported that the relationship between a_w and temperature varied widely among different low-moisture products. When the temperature increased from 25 to 80°C, the a_w of wheat flour increased from 0.45 to 0.80 a_w, but the peanut butter a_w decreased from 0.45 to 0.04 a_w. The $D_{80°C}$ of *Salmonella* in wheat flour and peanut butter was 6.9 and 17 min, which corresponds with a_w changes at high temperature. Therefore, these a_w effects likely have an impact on the tested and reported thermal resistance of *Salmonella*.

2.5.3 Primary model

Various mathematical models have been developed to describe microbial growth and inactivation processes. During thermal processing, the purpose of the inactivation model is to understand and predict the thermal resistance and survival of bacteria (McKellar and Lu, 2004).

The first-order, log-linear model is a well-known primary model that describes first-order reaction kinetics in heat processing. Log-linear inactivation kinetics have been used to estimate D-values (time required for a log reduction), by the following equation:

$$\log \frac{N}{N_0} = -\frac{t}{D(T)} \quad (2)$$

where N and N_0 are the bacterial populations (CFU/g) at times t and 0, respectively; t is the period of time of the isothermal treatment; and $D(T)$ is the time (min) required to reduce the microbial population by 90% (1-log reduction) at a specified temperature (McKellar and Lu, 2004).

However, some studies of thermal inactivation in low-moisture foods reported survival curves that did not follow log-linear kinetics (Abd et al., 2012; Ma et al., 2009). In those cases, the Weibull model has been applied as shown below:

$$\log \frac{N}{N_0} = -\left(\frac{t}{\delta}\right)^p \quad (3)$$

where N and N_0 are the populations (CFU/g) at times t and 0, respectively; t is the time of the isothermal treatment; p is the shape factor, and δ is the location factor (Peleg, 2006).

2.5.4 Secondary models

Secondary models have been developed to account for the effects of environmental factors such as temperature, pH, and product a_w, on primary model parameters (Gaillard et al., 1998). For

this research, product structure, water activity, and temperature are factors that may impact *Salmonella* thermal resistance; therefore, the secondary model will be applied to evaluate the combined effects of temperature, water activity, and product structure.

However, product structure is not a continuous variable that can be applied within secondary models. The Bigelow-type model is a common secondary model that has been used to describe the effects of temperature and water activity on the D-value. The Bigelow-type model is based on the model structure of Gaillard et al. (1998) and can be written as:

$$\log D_{T,a_w} = \log D_{ref} - \left(\frac{T - T_{ref}}{z_T}\right) - \left(\frac{a_w - a_{w,ref}}{z_{a_w}}\right) \quad (4)$$

where D_{ref} is the time required to reduce the microbial population by 90% (1 log reduction) at $T=T_{ref}$ and $a_w = a_{w,ref}$; T is temperature (°C); T_{ref} is the optimized reference temperature (°C); $a_{w,ref}$ is the optimized reference water activity (a_w is between 0 to 1); z_T and z_{aw} are temperature (°C) and water activity changes required for increasing or decreasing the D-value by a log cycle.

2.5.5 Model selection

Error measurements have been used to evaluate the appropriateness and effectiveness of models. Model errors can be described by the coefficient of determination (R^2) or, root mean squared error (RMSE):

$$R^2 = 1 - \frac{\sum(\log N_{predicted} - \log N_{observed})^2}{\sum(\log N_{predicted} - \log N_{average})^2} \quad (5)$$

$$RMSE = \sqrt{\frac{\sum(\log N_{predicted} - \log N_{observed})^2}{n-m}} \quad (6)$$

where $N_{predicted}$ and $N_{observed}$ are the bacterial populations (CFU/g) at predicted and observed times; $N_{average}$ is the average population (CFU/g) from time 0 to t; n is the number of observation points; and m is the number of model parameters.

Additionally, models applied to a single data set can be compared via the Corrected Akaike Information Criterion (AIC_c) (Motulsky and Christopoulos, 2004):

$$AIC_c = n \times ln\left(\frac{SS}{n}\right) + 2K + \frac{2K(K+1)}{n-K-1} \quad (7)$$

where n is the number of data; SS is the sum of squared residuals; and K is the number of parameters plus 1. A lower AIC_c indicates the more-likely-correct model.

2.5.6 Modeling Salmonella inactivation in low-moisture foods

Models for thermal inactivation of bacteria in low-moisture foods have been developed for specific conditions, such as sucrose solution effects of a_w on the thermal inactivation of *Listeria* monocytogenes (Sanchez-Zapata et al., 2011), the inactivation kinetics of *Salmonella* Enteritidis PT 30 on ground almond kernels under dry conditions (Villa-Rojas et al., 2013), or the combined effects of temperature, pH, and water activity on heat resistance of *Bacillus* cereus spores (Gaillard et al., 1998).

A_w is one of the most influential factors used in model development for low-moisture foods. Smith and Marks (2015) assessed the thermal resistance of *Salmonella* Enteritidis PT 30 on wheat flour subjected to rapid a_w changes (Smith and Marks, 2015). In this study, the a_w of the samples was rapidly decreased from 0.60 a_w to 0.30 a_w or increased from 0.30 a_w to 0.60 a_w. A log-linear model was used to estimate the parameters that described *Salmonella* inactivation. However, for each of the models (0.60 a_w to 0.30 a_w and 0.30 a_w to 0.60 a_w), the R^2 values were low (0.42

and 0.73), respectively. The study by Smith et al. (2016) also supports the importance of water activity by evaluating thermal resistance of *Salmonella* Enteritidis PT 30 on wheat flour with primary (log-linear and Weibull type) and secondary models (second-order response surface, modified Bigelow, and combined effects) . The log-linear and the modified Bigelow-type models were the best models, based on RMSE and AIC_c values.

Mattick et al. (2001) reported the heat tolerance of *Salmonella* serovars at 50 to 80°C and water activities of 0.65 to 0.90 a_w using a Weibull model. Secondary inactivation models were evaluated by comparing regression coefficients and analyzing P values. However, the generated thermal inactivation models underpredicted the thermal death rate in low-moisture foods, suggesting that additional factors should be included.

Villa-Rojas et al. (2013) used a polynomial secondary model to assess the effect of temperature and a_w on *Salmonella* thermal inactivation. The first-order kinetics model had good correlation coefficients (0.82 to 0.92), but the Weibull model was better (0.93 to 0.99). Use of Mafart's modified Bigelow model as a secondary model resulted in a good fit for both D and δ (R^2 = 0.927 and 0.818). Therefore, the thermal inactivation of *Salmonella* Enteritidis PT 30 on almond kernels could be described by the Weibull distribution model.

Santillana-Farakos et al. (2013) evaluated the log-linear model, Geeraerd-tail model, Weibull model, Biphasic-linear model, and Baranyi model by using the F-value, the root mean squared error (RMSE), and the adjusted coefficient of determination (R^2_{adj}). The Weibull model best described *Salmonella* survival kinetics in low-moisture foods. Additionally, Santillana-Farakos et al. (2013) developed a secondary model for predicting the effects of a_w, water mobility, and temperature on the survival of *Salmonella* in whey protein powder. This secondary model was

also evaluated for other low-moisture foods, such as cereal, nuts, and peanut butter (Santillana-Farakos et al., 2014b).

Li et al. (2014a) also used the Weibull model to assess *Salmonella* thermal inactivation in foods of modified composition - peanut butter and peanut butter spread. Results suggested that the effect of temperature can be described by a log-linear model, and the survival curves can be described by the Weibull model.

2.6 Conclusion

The impact of the inoculation procedure on *Salmonella* thermal resistance for fabricated products (such as powders and pastes) has already been demonstrated, but has never been evaluated with differing inoculation steps (before and after fabrication process). Factors, such as water activity, product structure, and temperature, have an impact on *Salmonella* thermal resistance in low-moisture foods, and must also be evaluated to understand bacterial behavior during thermal pasteurization processes. Based on the overall literature review, this dissertation represents the first study known to quantify and report how product structure affects the thermal resistance of *Salmonella.* Lastly, the evaluation of *Salmonella* thermal resistance during long-term storage periods will be important to confirm the relevance of thermal inactivation parameters to real-world process validations.

Ultimately, thermal inactivation models can be used to improve food safety validation methods for low-moisture foods. Moreover, the behavior of *Salmonella* in low-moisture foods, at different temperatures, product structures, and water activities, can be used to improve current inactivation processes and process validation methodologies.

3 INOCULATION PROTOCOLS INFLUENCE THE THERMAL RESISTANCE OF *SALMONELLA* ENTERITIDIS PT 30 IN FABRICATED ALMOND, WHEAT, AND DATE PRODUCTS

Inoculation methods representing two contamination scenarios were assessed. Surface contamination can occur before, after, or even during processing and fabrication of low-moisture products. This experiment was designed to quantify the effect of inoculation protocol (pre- and post-fabrication) on the thermal resistance of *Salmonella* Enteritidis PT 30 in fabricated low-moisture foods (almond, wheat, and date products). This chapter was accepted for publication by the *Journal of Food Protection*.

3.1 Materials and Methods

Overall, the experimental design consisted of inoculating almond meal, almond butter, wheat meal, wheat flour, and date paste via two different inoculation protocols (pre-fabrication and post-fabrication). Thereafter, the thermal resistances of *Salmonella* in these samples were compared by performing isothermal heat treatments in triplicate. In general terms, the pre-fabrication protocols entailed inoculation of intact natural products (i.e., whole almond kernels, wheat kernels, and date pieces), which would correspond to environmental, in-field, or preprocessing contamination, and then those products were processed to produce meal, flour, butter, or paste. In contrast, the post-fabrication protocols entailed inoculation of the fabricated products after they were already produced, which would correspond to an in-plant or postprocessing contamination event. All known prior thermal inactivation studies with fabricated low-moisture products have been conducted using post-fabrication inoculation protocols.

3.1.1 Almond meal and almond butter

Almonds (Nonpareil, size 27/30, Select Harvest, Turlock, CA) were sourced from a retail supplier, vacuum-packed (350 g per bag), and stored at ~2.5°C for up to a year. Almond meal and almond butter were fabricated using a food processor (model FP21, Hamilton Beach Brands, Inc., Glen Allen, VA). To produce almond meal, whole almonds (100 g) were ground at the lowest speed setting for 45 s and sieved through US standard sieves no. 20 and 80 (W.S. Tyler, Inc., Mentor, OH), capturing the material between the two sieves as the meal. Almond butter was produced by similarly grinding 200 g of almonds for 15 min total, while adding dry ice pellets (~30 mL) every 2 min to maintain product temperature below 40°C (confirmed via a handheld infrared thermometer, model 566, Fluke Corporation, Everett, WA).

3.1.2 Wheat meal and wheat flour

Organic soft white whole wheat kernels (*Triticum aestivum*, Eden Foods Inc., Clinton, MI) were stored in their original package at room temperature (~20°C) for up to 6 months. Wheat meal and wheat flour were fabricated by milling whole wheat kernels (50 g) for 45 s in a coffee mill (model 501, Jura-Capresso Inc., Montvale, NJ). Fabricated wheat samples were sieved through US standard sieves no. 20, 80, and 200. Ground product passing through a no. 20 sieve, but not through a no. 80 sieve, was called wheat meal, whereas ground product passing through a no. 80 sieve, but not through a no. 200 sieve, was termed wheat flour.

3.1.3 Date paste

Dates (medjool, jumbo) were purchased from a retail supplier (Nuts.com, Cranford, NJ) and stored in their original package at ~2.5°C for up to a year. Date paste was fabricated by feeding

dates through a meat grinder plate with holes 1 cm in diameter (model K5-A, KitchenAid, Benton Harbor, MI). The resulting paste was then fed through the grinder two more times to ensure homogeneity, which was determined by sampling inoculated date paste and enumerating for *Salmonella* survivors in five subsamples per replication (~1 g each).

3.1.4 Inoculation and equilibration

The general inoculation preparation method was derived from the procedures of Danyluk et al. (Danyluk et al., 2005). *Salmonella* enterica serovar Enteritidis PT 30, previously obtained from Dr. Linda Harris (University of California, Davis), was kept frozen at -80°C in a concentrated culture containing 20% glycerol. The frozen culture was subjected to two successive 24 h (37°C) transfers in TSB (Difco, BD, Franklin Lakes, NJ) 17% (m/m) containing 0.6% yeast extract (Difco, BD). Thereafter, a plate (150 by 15 mm) of Trypticase soy agar (TSA; Difco, BD) containing 0.6% yeast extract (TSAYE) was spread for confluent growth and incubated for 24 h (37°C).

For pre-fabrication inoculation of almond and wheat products, the lawn cultures were each harvested in 10 mL of 0.1% peptone water. Thereafter, 8 mL of the liquid suspension (~$10^{7.5}$ to 10^9 CFU/mL) was added directly to 100 g of either almond or wheat kernels and mixed in a sterile plastic bag for 1 min. These wet inoculated samples were placed on filter paper (P8, Fisher Scientific, Pittsburgh, PA) in an open plastic container, dried (~3 h) in a biosafety cabinet, and then placed in an equilibration chamber (described in the ‘‘Equilibration’’ section) until they reached the target a_w (0.40 ± 0.02). After equilibration, the samples were processed into meal, flour, or butter and were re-equilibrated as described below.

For post-fabrication inoculation of almond and wheat samples, the *Salmonella* inocula (8 mL, grown and harvested as described above) were pelleted by centrifugation (model Sorvall RC 6 plus, SS-34 rotor, Thermo Fisher Scientific, Waltham, MA) at 2,988 × *g* for 15 min. To minimize the change in a_w during inoculation (and to prevent physical changes caused by the addition of water to the meals and powder), the *Salmonella* pellet was introduced into 50 g of almond meal, almond butter, wheat meal, or wheat flour and hand-mixed for 3 min in a sterile 24-oz (710-mL) plastic bag (Nasco, Fort Atkinson, WI). Inoculated samples then were equilibrated (as described below) until they reached the target a_w (0.40 ± 0.02).

In the pre- and post-fabrication protocols, date samples were inoculated using cell pellets that were produced by the same method as the post-fabrication protocol (described above) for almond and wheat samples. Based on preliminary tests, the inoculum was nonhomogeneous distributed by directly introducing the pellet into the date paste, because the highly viscous or semisolid structure of the paste impeded uniform distribution of the solid pellet. Therefore, the pellets were resuspended in 2 mL of 0.1% peptone water and homogenized using a vortex (model G-560, Scientific Industries Inc., Bohemia, NY). This highly concentrated suspension for inoculation contained ~10^{11} CFU/mL.

For pre-fabrication inoculation, whole dates were each cut into 12 pieces (~1.8 g each) for faster equilibration. Each date piece was spot-inoculated (200 µL of total inoculum across 12 pieces) on the date skin, dried for ≥20 min in a biosafety cabinet, and then conditioned to ~0.45 a_w in an equilibration chamber (described below) for up to 1 week. Date paste was fabricated by grinding the inoculated date pieces, as previously described. If the a_w after grinding was not 0.45

± 0.02, the paste was returned to the chamber and re-equilibrated to the target a_w (0.45). However, if the number of days the product spent re-equilibrating as paste exceeded the number of days spent originally equilibrating as inoculated pieces, the product was considered unusable and was discarded, in order to control the overall treatment for both the intact dates and paste.

For post-fabrication inoculation, dates were passed once through the grinder (previously described), after which 600 µL of the concentrated *Salmonella* suspension was added to 60 g of ground dates. The inoculated date paste was then passed through the grinder four more times to evenly distribute the inoculum prior to equilibration to 0.45 ± 0.02 a_w in the equilibration chamber.

3.1.5 Equilibration

Samples were placed in custom-designed equilibration chambers (Smith and Marks, 2015) to adjust and control the sample a_w. Controlled-humidity air (± 0.2%) obtained by mixing air passed through a desiccant column (dry air) or a water column (wet air) was monitored and controlled by a humidity sensor (DHT 22, Adafruit Industries, New York, NY) and a microcomputer. Batches of samples (~300 g of almonds, 100 g of wheat, and 50 g of dates) were equilibrated to 0.40 ± 0.02 (almonds and wheat) or 0.45 ± 0.02 (dates) a_w. Total equilibration times were 6-9 days for the almond meal, wheat meal, and wheat flour, and 11-14 days for the almond butter and date paste.

3.1.6 Water activity measurement

Water activity of representative samples (pulled after mixing the bulk inside the equilibration chamber) was measured daily using a water activity meter (AquaLab 3TE, Decagon Devices, Pullman, WA) to confirm that the target a_w was reached.

3.1.7 Thermal treatment

After equilibration to the target a_w, samples (~0.7 g of almond meal, 1.2 g of almond butter, 0.6 g of wheat meal, 0.5 g of wheat flour, and 1.2 g of date paste) were loaded into sealed aluminum test cells (Chung et al., 2008) in the equilibration chamber to prevent a_w changes. Sample thickness in the aluminum test cells was less than 1 mm. Samples were heated in an isothermal water bath set at 80.5°C (GP-400, Neslab, Newington, NH). Come-up time for the product to reach the target temperature (79.5°C) was measured in six replicates for each sample type, using a test cell with a T-type thermocouple probe positioned at the geometric center of the sample, and was averaged for use in all further experiments. After reaching the come-up time (2.0 ±0.1 min for almond meal, 2.8 ±0.1 min for almond butter, 1.3 ±0.1 min for wheat meal, 1.4 ±0.3 min for wheat flour, and 2.5 ±0.1 min for date paste), the initial (time zero) sample was removed, and subsequent samples were pulled at pre-determined time points and immediately cooled in an ice bath to halt further bacterial inactivation.

3.1.8 Recovery and enumeration

Samples were aseptically removed from the test cells, diluted (1:10 dilution) in 0.1% peptone water, and homogenized by stomaching for 3 min (Model 1381/471, NEU-TEC Group Inc., Farmingdale, NY). Serial dilutions in 0.1% peptone water were plated in duplicate on mTSAYE (TSAYE supplemented with 0.05% of ammonium ferric citrate and 0.03% of sodium thiosulfate pentahydrate; Fisher Chemical, Fair Lawn, NJ), which was a non-selective differential medium. The plates were incubated for 48 h at 37°C prior to counting the black colonies as *Salmonella*. Preliminary tests with uninoculated samples yielded no such colonies for any of the materials used in this study.

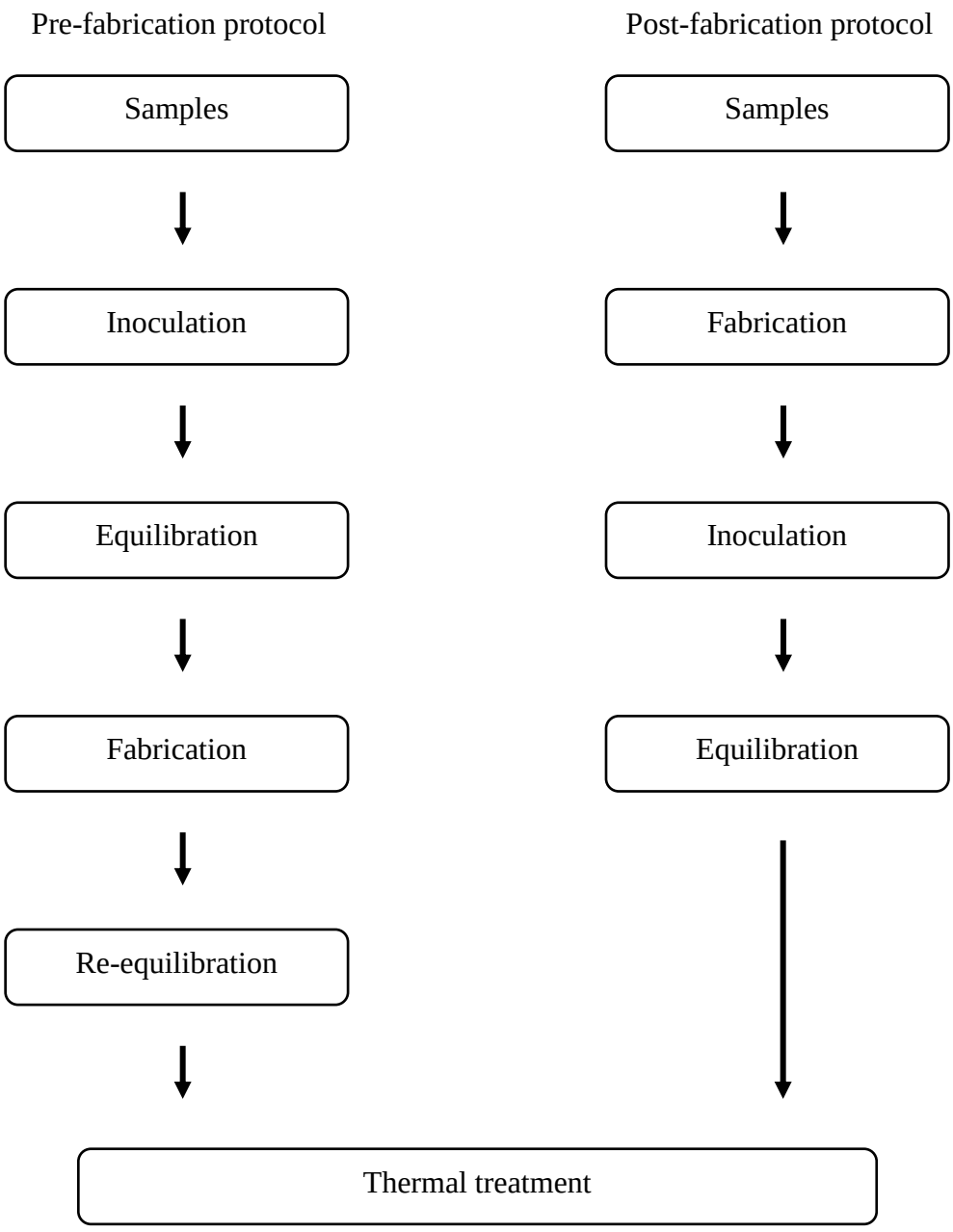

Figure 3.1 Inoculation steps for pre- and post-fabrication protocols.

3.1.9 Statistical analyses

Initial *Salmonella* populations and initial a_w values from the pre-fabrication and post-fabrication methods were compared using the paired t-test (Microsoft Excel 2013 software, Microsoft Inc., Seattle, WA). For the pre-fabrication method, a_w and *Salmonella* populations on the initial inoculated samples (kernels/fruits) and final samples (meal/butter/paste/flour) also were compared via a paired t-test.

Reproducibility for each product was determined by calculating the standard error of replication as follows:

$$\sigma_{rep} = \sqrt{\frac{\sum_{j=1}^{m}\sum_{i=1}^{n}(y_{ij}-\bar{y}_i)^2}{m \cdot n - m}} \quad (8)$$

where *m* is the number of data points over time for each survival curve, *n* is the number of replications for each observation point, and *y* is the *Salmonella* population (log CFU/g).

After pooling all triplicate data (Appendix A) within each treatment, the inactivation model parameters were estimated using nlinfit (nonlinear regression routine in the statistical toolbox) in MATLAB (version R2016a, MathWorks Inc., Natick, MA) for the log-linear and Weibull models. The log-linear model was estimated by the following equation:

$$\log\frac{N}{N_0} = -\frac{t}{D(T)} \quad (9)$$

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), and $D(T)$ is the time (min) required to reduce the microbial population by 90% at a specified temperature (T, °C).

The Weibull model parameters were estimated, according to the following equation (Peleg, 2006):

$$\log \frac{N}{N_0} = -\left(\frac{t}{\delta}\right)^p \tag{10}$$

where p is the shape factor, and δ is the location factor (min). The estimated time for a 1 log-reduction (min) in each sample was calculated by the following equation (van Boekel, 2002):

$$t = \delta \cdot (-\ln(10^{-d})^{\frac{1}{p}}) \tag{11}$$

where d is the number of decimal reductions (i.e., d =1 for a 1 log reduction).

The Corrected Akaike Information Criterion (AIC_c) (Motulsky and Christopoulos, 2004) was calculated to select the most-likely-correct model, with the lower AIC_c indicating the more-likely-correct model:

$$AIC_c = n \cdot ln\left(\frac{SS}{n}\right) + 2K + \frac{2K(K+1)}{n-K-1} \tag{12}$$

where n is the number of data points; SS is the sum of squares of residuals, and K is the number of parameters plus 1. The relative probability of each model being the correct model also was calculated as follows (Motulsky and Christopoulos, 2004):

$$\text{Relative likelihood of loglinear over Weibull model} = \frac{e^{\left(\frac{AIC_{c,log-linear\ model} - AIC_{c,Weibull\ model}}{2}\right)}}{1+e^{\left(\frac{AIC_{c,log-linear\ model} - AIC_{c,Weibull\ model}}{2}\right)}} \tag{13}$$

Model parameters for pre- and post-fabrication samples of each product were also compared using the paired t-test (Microsoft Excel 2013).

3.2 Results and Discussion

3.2.1 Sample preparation and water activity control

For the pre-fabrication methods, *Salmonella* Enteritidis PT 30 populations on the products after fabrication (i.e., meal, butter, flour, paste) were not significantly different from the populations on the intact products prior to fabrication (i.e., almonds, wheat kernels, date pieces) ($P > 0.05$). Additionally, the pre- and post-fabrication products had similar a_w values ($P > 0.05$).

In a comparison of initial *Salmonella* Enteritidis PT 30 populations between the pre- and post-fabrication protocols before heating (Table 3.1), initial populations in date paste were statistically equivalent for the pre- and post-fabrication methods ($P > 0.05$, 7.6 to 7.7 log CFU per sample). Additionally, separate subsampling tests yielded good homogeneity for both date preparation methods (± 0.2 and ± 0.3 log CFU/g for pre- and post-fabrication, respectively).

Salmonella populations for almond and wheat products in the pre-fabrication method were significantly lower ($P < 0.05$) than those for the post-fabrication method (Table 3.1), because the *Salmonella* Enteritidis PT 30 concentration in the pellet inoculum for post-fabrication was higher than in the liquid inoculum for pre-fabrication. For date paste, the initial pre- and post-fabrication populations of *Salmonella* Enteritidis PT 30 were similar ($P > 0.05$) and were lower than the other product types because the inoculum contained fewer cells. However, prior results have shown that initial inoculation level does not affect thermal resistance of *Salmonella* Enteritidis PT 30 in low-moisture products (Hildebrandt et al., 2016); therefore, comparisons of thermal resistance between pre- and post-fabrication samples should not be affected by these differences in initial population.

Table 3.1 *Salmonella* population (± standard deviation) and water activity (± standard deviation) of almond meal, almond butter, date paste, wheat meal, and wheat flour subjected to pre-fabrication and post-fabrication inoculation protocols before heating

Products	*Salmonella* population (log CFU/g)		Water activity	
	Pre-fabrication Protocol	Post-fabrication Protocol	Pre-fabrication	Post-fabrication
Almond meal	8.0 ± 0.3 ^{A}	9.2 ± 0.2 ^{B}	0.410 ± 0.014 ^{A}	0.393 ± 0.003 ^{A}
Almond butter	7.7 ± 0.2 ^{A}	9.3 ± 0.3 ^{B}	0.414 ± 0.012 ^{A}	0.406 ± 0.004 ^{A}
Date paste	7.7 ± 0.2 ^{A}	7.6 ± 0.2 ^{A}	0.450 ± 0.015 ^{A}	0.456 ± 0.019 ^{A}
Wheat meal	8.8 ± 0.1 ^{A}	9.7 ± 0.1 ^{B}	0.406 ± 0.009 ^{A}	0.405 ± 0.005 ^{A}
Wheat flour	9.0 ± 0.1 ^{A}	9.7 ± 0.1 ^{B}	0.392 ± 0.017 ^{A}	0.400 ± 0.012 ^{A}

Within a row (and same measurement), means with a common superscript letter were not significantly different ($\alpha = 0.05$).

3.2.2 Model selection

Model parameters (Table 3.2) for the log-linear and Weibull models were estimated using *Salmonella* Enteritidis PT 30 survival data (Figure 3.2). AIC_c analysis (Table 3.2) gave the most-likely-correct model for each product type. The Weibull model was more likely correct for pre-fabrication almond meal (% likelihood > 99.99%), pre-fabrication almond butter (% likelihood > 99.99%), post-fabrication almond butter (% likelihood > 99.99%), pre-fabrication wheat meal (% likelihood > 90%), pre-fabrication wheat flour (% likelihood > 96%), and post-fabrication wheat flour (% likelihood > 84%). However, the log-linear model was more likely correct for post-

fabrication almond meal, pre- and post-fabrication date paste, and post-fabrication wheat meal (% likelihood, ~70 to 98%). Because the Weibull model was not the most-likely-correct model for all products and was dependent on product type and inoculation protocol, both the $D_{80°C}$ value and the Weibull-estimated time for a 1-log reduction were calculated and compared for all products (Table 3.2).

Table 3.2 Standard errors of replications, $D_{80°C}$ values (± standard error) determined by non-linear regression of the *Salmonella* survivor curves, and δ (± standard error) and p (± standard error) Weibull parameters for the almond meal, almond butter, date paste, wheat meal, and wheat flour (~0.40 – 0.45 a_w) subjected to pre-fabrication and post-fabrication inoculation protocols.

		Log-linear model			Weibull model				
Products	Standard error of replications (log CFU/g)	D-value (min)	RMSE (log CFU/g)	AIC_c	δ (min)	p	RMSE (log CFU/g)	AIC_c	Estimated time for one-log reduction (min)	Relative likelihood of log-linear over Weibull model (per AIC)
Almond meal										
Pre-fabrication	0.33	49.8 ± 2.1^{A}	0.418	-54.1	29.6 ± 4.5^{A}	0.61 ± 0.07^{A}	0.308	-72.9	29.6 ± 5.3^{A}	0.0001
Post-fabrication	0.85	33.4 ± 1.7^{B}	0.729	-18.6	34.1 ± 6.4^{A}	1.02 ± 0.15^{B}	0.740	-14.4	34.1 ± 6.8^{A}	0.8870
Almond butter										
Pre-fabrication	0.90	42.9 ± 2.6^{A}	0.694	-20.7	8.5 ± 3.5^{A}	0.37 ± 0.06^{A}	0.390	-57.4	8.5 ± 3.0^{A}	~0.0000
Post-fabrication	0.49	18.3 ± 1.0^{B}	1.132	2.0	4.7 ± 1.5^{A}	0.57 ± 0.06^{A}	0.477	-32.0	3.4 ± 0.9^{A}	~0.0000
Date paste										
Pre-fabrication	0.31	3.5 ± 0.5^{A}	0.322	-72.5	3.3 ± 0.6^{A}	1.11 ± 0.38^{A}	0.327	-66.8	3.3 ± 0.4^{A}	0.9436
Post-fabrication	0.79	1.2 ± 0.1^{B}	0.696	-20.5	1.1 ± 0.2^{B}	1.30 ± 0.37^{A}	0.699	-18.8	1.4 ± 0.3^{B}	0.6995
Wheat meal										
Pre-fabrication	0.80	10.3 ± 0.3^{A}	0.422	-59.7	5.8 ± 0.7^{A}	0.69 ± 0.05^{A}	0.279	-64.0	5.8 ± 0.8^{A}	0.1043
Post-fabrication	0.33	19.5 ± 0.8^{B}	0.652	-35.7	7.5 ± 1.6^{A}	0.60 ± 0.05^{A}	0.373	-42.3	7.5 ± 1.6^{A}	0.0367
Wheat flour										
Pre-fabrication	0.54	8.9 ± 0.4^{A}	0.619	-36.4	5.1 ± 1.2^{A}	0.71 ± 0.09^{A}	0.524	-28.6	5.1 ± 1.2^{A}	0.9802
Post-fabrication	0.74	15.1 ± 0.7^{B}	0.978	-7.6	4.7 ± 1.7^{A}	0.59 ± 0.08^{A}	0.726	-10.9	4.7 ± 1.4^{A}	0.1572

Within a column (and within the same product), means with common superscript letters were not significantly different (α = 0.05).

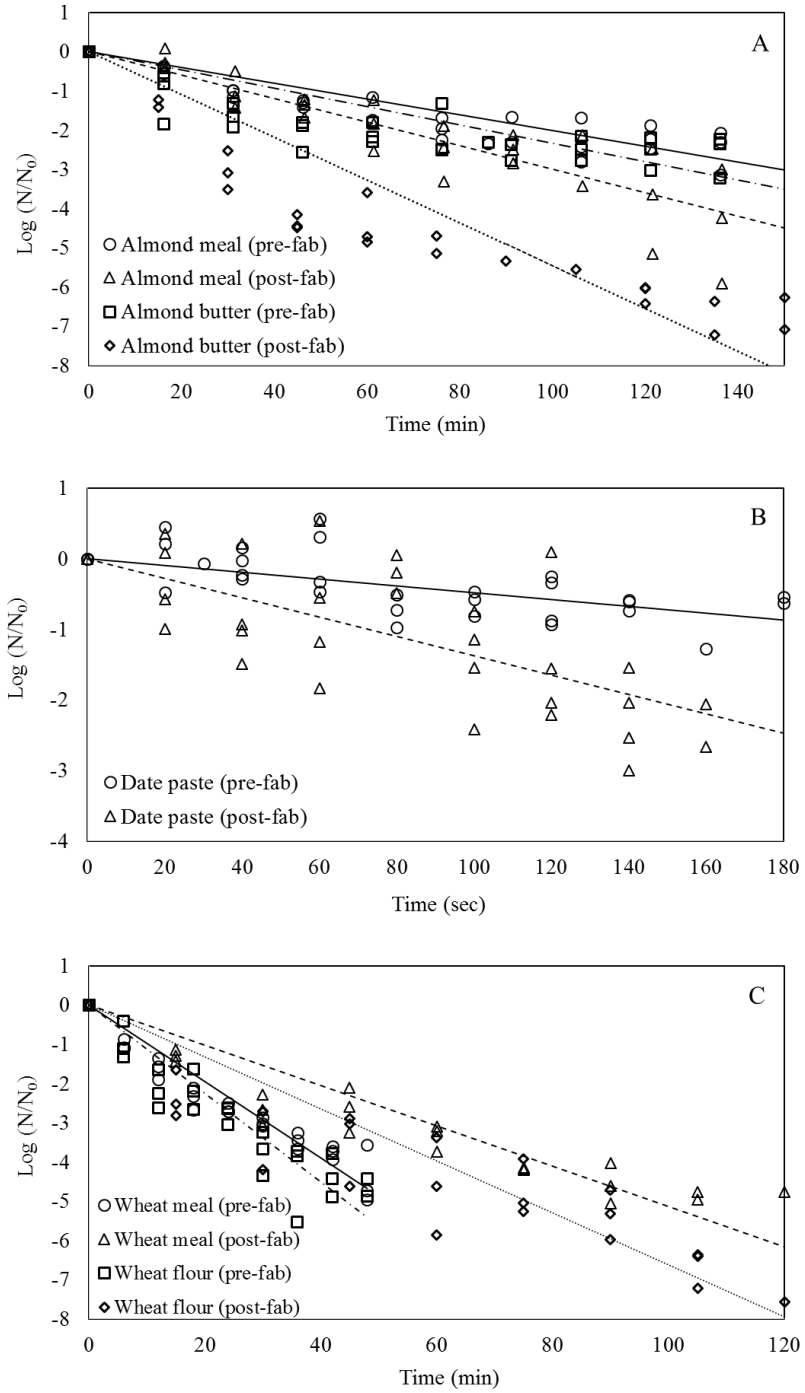

Figure 3.2 Isothermal (80°C) *Salmonella* survival curves and log-linear model fit after pre-fabrication and post-fabrication inoculation of: (A) almond meal and almond butter at 0.40 a_w, (B) date paste at 0.45 a_w, and (C) wheat meal and wheat flour at 0.40 a_w.

3.2.3 Replication error

Replication errors (Table 3.2) for each product were calculated to quantify consistency of the experiments. The highest standard error of replication (0.90 log CFU/g) was for pre-fabrication almond butter, which may have been affected by oil separation during the equilibration process.

3.2.4 Product effects

Based on the pre-fabrication $D_{80°C}$ values, *Salmonella* Enteritidis PT 30 thermal resistance in almond products was approximately four times greater ($P < 0.05$) than in wheat products, which was approximately three times greater ($P < 0.05$) than in date products. For the post-fabrication results, the same general rank ordering was true ($P < 0.05$), except for a smaller difference between almond and wheat products. This observation is consistent with prior *Salmonella* Enteritidis PT 30 studies, which have reported larger D-values for high fat products (e.g., $D_{83°C}$ of 16 min for peanut butter (Ma et al., 2009) as compared to a $D_{80°C}$ of 5 min for wheat flour (Smith et al., 2016).

3.2.5 Structure effects

Salmonella Enteritidis PT 30 thermal resistance was significantly greater in almond butter than in almond meal ($P < 0.05$) for the post-fabrication protocol. In addition, *Salmonella* Enteritidis PT 30 thermal resistance in wheat meal was significantly ($P < 0.05$) greater than in wheat flour for both inoculation protocols. Surface interactions between product particles and *Salmonella* cells during fabrication may have impacted *Salmonella* Enteritidis PT 30 attachment differently in almond and wheat products (due to significantly different composition between these products), resulting in different impacts on thermal resistance; however, the fundamental mechanisms causing these differences are not yet conclusively known.

3.2.6 Almond products

The $D_{80°C}$ for pre-fabrication almond meal (49.8 min) was higher ($P < 0.05$) than that for post-fabrication almond meal (33.4 min). Villa-Rojas et al. (2013) reported a much lower $D_{80°C}$ of 1.63 min for almond meal at 0.60 a_w compared to this study, which would be expected to be due to the differences in a_w. Additionally, this may have been impacted by differences in inoculum preparation, in that the prior study used phosphate buffer as the liquid suspension.

In almond butter, the pre-fabrication $D_{80°C}$ for *Salmonella* Enteritidis PT 30 (42.9 min) was two times greater than for post-fabrication (18.3 min). During the milling process, almond oil was expressed, and bacteria were presumably forced into the oil droplets. It can be assumed that the internal shear force during hand mixing (post-fabrication) was much lower than for mechanical stomaching (pre-fabrication); therefore, the fraction of *Salmonella* Enteritidis PT 30 cells entrained in the oil phase likely increased during fabrication, leading to greater thermal resistance in pre- as opposed to post-fabrication almond butter. This enhanced survival is supported by the published literature indicating that high fat content protects bacterial cells at high temperature (Shachar and Yaron, 2006).

Thermal resistance of *Salmonella* has been assessed in peanut butter, but not in almond butter. Based on the log-linear model, Ma et al. (2009) and He et al. (2011) and (2013) reported a $D_{83°C}$ of *Salmonella* Tennessee in regular peanut butter of 16 min at 0.45 a_w, and a $D_{90°C}$ for a *Salmonella* cocktail on regular and low-fat peanut butter of 3.5 and 2.6 min, respectively, at 0.40 a_w. Therefore, *Salmonella* strain, temperature, and fat content can be assumed to affect thermal resistance of *Salmonella* in nut butter products during processing (He et al., 2011; Ma et al., 2009; Shachar and Yaron, 2006).

The Weibull distribution also has been previously used to model *Salmonella* inactivation in peanut butter. Ma et al. (2009) and He et al. (2013) reported the Weibull parameters and estimated times for 1 log-reduction of 1.92 min at 83°C, and 6.62 min at 90°C. Weibull parameters from Li et al. (2014a) yielded an estimated time for one log-reduction (80°C) of a *Salmonella* cocktail (Thompson, Newport, Typhimurium, Copenhagen, Montevideo, and Heidelberg) in regular peanut butter (0.45 a_w) of 1.9 min, which was lower than in pre-fabrication (8.5 min) and in post-fabrication (3.4 min) almond butter in this study.

3.2.7 Date products

Thermal resistance of *Salmonella* Enteritidis PT 30 in post-fabrication inoculated date paste was the lowest amongst all the products ($D_{80°C}$ ~1.2 min). *Salmonella* cells originally inoculated onto the date surface (pre-fabrication protocol) were more thermally resistant than those inoculated directly into the date paste (post-fabrication protocol). In the pre-fabrication method, the inoculated dates were equilibrated before grinding and re-equilibrated after grinding, but the post-fabrication samples were equilibrated in paste form. This difference in equilibration procedures, necessitated by the different fabrication procedures, may partially explain the observed differences in *Salmonella* Enteritidis PT 30 thermal resistance.

Date paste also has a very high sugar content (~66%) (U.S. Department of Agriculture, 2016). Although previous studies on *Salmonella* thermal resistance in date paste are lacking, Mattick et al. (2001) reported the Weibull parameters for high sugar content broths (0.65 a_w) at 80°C. Their estimated time for a 1-log reduction of *Salmonella* Typhimurium was 3.6 min, which was higher than that for post-fabrication inoculated date paste (1.5 min) in this study, but on the same order of magnitude.

3.2.8 Wheat products

Thermal resistance of *Salmonella* Enteritidis PT 30 in wheat meal and wheat flour showed an opposite result from the almond and date products, with resistance greater in post- as opposed to pre-fabrication samples. In the pre-fabrication protocol, wheat meal and flour particle surfaces that previously were internal in the intact wheat kernel would have been cross-contaminated from the inoculated external surfaces during grinding and handling. However, in the post-fabrication protocol, all wheat meal and flour particle surfaces had equal probability of being contaminated when the inoculum was added to the powders and mixed. This difference between the two protocols therefore may have influenced the extent of *Salmonella* Enteritidis PT 30 attachment to any given particle surface, which could have affected thermal resistance in a manner that would have been different than in the almond products, given the significantly different compositions.

According to Smith et al. (2016), *Salmonella* Enteritidis PT 30, which was inoculated via a similar method as the present post-fabrication protocol, exhibited a $D_{80°C}$ of 5.5 min in wheat flour at 0.43 a_w, which was lower than that for post-inoculation wheat flour at 0.4 a_w (15.1 min). They also used commercial white wheat flour, which may have altered the heat resistance, due to differences in composition (i.e., lower lipids content) and particle-cell interactions (Smith et al., 2016). Syamaladevi et al. (2016a) also assessed thermal inactivation of a *Salmonella* cocktail in wheat flour at 80°C (inoculated post-fabrication). At 0.45 a_w, the $D_{80°C}$ was 6.9 min, which was lower than for the post-fabrication method used in this study (15.1 min). The Syamaladevi et al. (2016a) experiment was similar to this study, except for the inoculum preparation. These results support the premise that inoculation procedures impact thermal resistance of *Salmonella* in wheat flour (Hildebrandt et al., 2016).

3.3 Conclusion

The results have shown that thermal resistance of *Salmonella* Enteritidis PT 30 depends on the inoculation protocol, product type, and product structure. In all known prior studies with fabricated products (e.g., peanut butter (He et al., 2013; Ma et al., 2009), wheat flour (Hildebrandt et al., 2016; Smith and Marks, 2015), and dried fruits (Beuchat and Mann, 2014)), post-fabrication inoculation protocols were applied to inoculate products, determine inactivation kinetics, and validate the processes. This suggests that some published data may not accurately reflect actual scenarios where a raw material is contaminated and then fabricated into an ingredient or finished product, which may influence thermal resistance. These results also suggest that pre-fabrication contamination events may be of greater concern in process validation. Additional tests are being conducted to quantify *Salmonella* thermal resistance in different product matrices at various a_w levels and to model *Salmonella* behavior in a range of low-moisture foods.

4 SURVIVAL AND THERMAL RESISTANCE OF *SALMONELLA* ENTERITIDIS PT 30 ON ALMONDS AFTER LONG-TERM STORAGE

Salmonella in low-moisture foods can survive for long periods. However, the thermal resistance of *Salmonella* on almonds after long-term storage has been reported for only one thermal process (hot oil treatment). In this study, the effects of long-term storage on the survival and thermal resistance of *Salmonella* Enteritidis PT 30 on almonds were evaluated.

4.1 Materials and Methods

4.1.1 Almond kernels

Nonpareil almond kernels (size 27/30, Select Harvest, Turlock, CA) were vacuum packaged (350 g/bag) and stored at ~2.5°C.

4.1.2 Inoculation preparation

The Danyluk et al. (Danyluk et al., 2005) inoculation procedure was followed with slight modifications described below. *Salmonella enterica* serovar Enteritidis phage type 30 (obtained from Dr. Linda Harris, University of California, Davis) was stored at -80°C in Trypticase Soy Broth (TSB; Difco, BD, Franklin Lakes, NJ) supplemented with 20% (vol/vol) glycerol. The original culture was transferred to a tube of TSB containing with 0.6% yeast extract (TSYBE) (Difco, BD) for 24 h (37ºC), transferred to another tube of TSYBE and incubated for an additional 24 h (37ºC), and then transferred to a plate (150 by 15 mm) of Trypticase Soy Agar (TSA; Difco, BD) containing 0.6% yeast extract (TSAYE) to obtain confluent growth after 24 h (37ºC). The lawn culture was harvested using 10 ml of 0.1% peptone water (Buffered Peptone Water; Difco,

BD) per lawn plate for 5 plates in totals, and the inoculum was collected in a sterile plastic bottle before inoculating the almond kernels.

4.1.3 Almond inocualtion

Prior to inoculation, the refrigerated almond kernels were held at room temperature for 30 min. The almonds (500 g) were hand-mixed with 40 ml of the inoculum (~$10^{7.5}$ to 10^{9} CFU/ml) in a sterile plastic bag for 1 min, removed and placed in a single layer on filter paper (P8, Fisher Scientific, Pittsburgh, PA), and dried for ~3 h in a biosafety cabinet before being moved into a humidity-controlled equilibration chamber.

4.1.4 Water activity equilibration

Custom-designed equilibration chambers were used to maintain the humidity conditions during equilibration of the inoculated almonds prior to long-term storage and thermal treatment (Smith and Marks, 2015). The humidity (45 ± 0.2%) was maintained by passing air through either a dry column (desiccant beads) or wet column (DI water), monitoring the chamber with a humidity sensor (DHT 22, Adafruit Industries, New York, NY), and controlling the mix via solenoid values controlled by a microcomputer (Arduino Mega 2560, Turin, Italy). In the chamber, the almonds were spread in a single layer on perforated metal shelves and equilibrated for ~7 days to 0.45 ± 0.02 $a_{w,}$ which was confirmed by a water activity meter (AquaLab 4TE, Decagon Devices, Pullman, WA).

4.1.5 Long-term storage

Inoculated almonds from the same batch were randomly separated into two groups (I and II) of 250 g each, placed into steel cans (16 oz., Uline, Pleasant Prairie, WI), sealed with electrical tape (3M Co., Ltd, Two Harbors, MN), and stored in an insulated container at room temperature (23 ± 0.2°C). Group I subsamples were removed at 0, 7, 15, 27, and 68 weeks to quantify *Salmonella* survival and thermal resistance (described below). Group II subsamples were removed at 70 and 103 weeks for the same analyses. Each group consisted of samples from three different initial inoculations. For group I, after each storage period, a random subsample was removed from each replicate to measure a_w. If the a_w was out of the target range for testing (0.45 ± 0.02 a_w), the entire group I sample was unpacked from the storage container and placed in the equilibration chamber (5-7 days) until the target a_w was achieved. Then, a subsample (~15 g/replicate) was randomly removed for the thermal inactivation test, and the remaining unused almonds were placed back into storage as described above. For group II, the almonds remained in the sealed steel cans, which were not opened until weeks 70 and 103. The group II samples were tested using the same methodology as group I, but they were not re-equilibrated in the chambers prior to thermal treatment.

4.1.6 Thermal treatment

Single almonds were vacuum-packaged as a thin layer (< 1 mm) in plastic bags (4 oz., Nasco, Fort Atkinson, WI), with a total of 9 bags per replicate (1 bag for 1 experimental time-point in each treatment). Before performing the thermal inactivation experiments, the thermal come-up time was established by inserting thin-wire thermocouples (T-type, 36 gauge, OMEGA Engineering Inc., Stanford, CT) underneath the skin of six replicates of individually vacuum-

packaged almonds, to determine the time for the almond surface temperature to reach within 0.5°C of the 80°C target temperature in a water bath (GP-400, Neslab, Newington, NH). For the experiments, the initial (time zero) samples were removed from the water bath after the come-up time (2.7 ± 0.4 min) had been reached. Subsequently, almonds were removed at 8 additional time points up to 96 min of heating, and the bags were immediately submerged in an ice bath for >1 min.

4.1.7 Enumeration

The cooled samples were aseptically unpacked and diluted (1:10) in 0.1% peptone water, stomached for 3 min (Model 1381/471, NEU-TEC Group Inc, Farmingdale, NY), serially diluted, and plated on modified TSAYE supplemented with 0.05% of ammonium ferric citrate and 0.03% of sodium thiosulfate pentahydrate (Fisher Chemical, Fair Lawn, NJ). The *Salmonella* survivors (differentiated by black colonies) were enumerated after incubating for 48 h at 37°C. *Salmonella* survivor data points were omitted if the average count of the duplicate plates was not within 25-250 colonies (Tomasiewicz et al., 1980).

4.1.8 Statistical analyses

The *Salmonella* survival data from groups I and II were compared within each group. The variation in a_w and *Salmonella* survival (log CFU/g) during storage was evaluated by analyses of variance (ANOVA) with Tukey means comparison, using Minitab (version 18, Minitab Inc., State College, PA). Survivor data at the different storage periods were also compared by using analysis of covariance (ANCOVA) in MATLAB within a group.

Additionally, log-linear and Weibull models were fit to pooled triplicate survivor data (Appendix B) by using nlinfit (nonlinear regression routine in the statistical toolbox) in MATLAB (version R2016a, MathWorks Inc., Natick, MA).

The log-linear model parameters were estimated by using the following equation;

$$\log N = -\frac{t}{D(T)} + \log N_0 \tag{14}$$

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); and $D(T)$ is the time (min) required to reduce the microbial population by 90% at a specified temperature (T, °C).

The Weibull model parameters were estimated according to the following equation (Peleg, 2006):

$$\log \mathrm{N} = -\left(\frac{t}{\delta}\right)^{p} + \log N_0 \tag{15}$$

where p is the shape factor, and δ is the location factor (min).

The Weibull model parameters were also compared (for different storage time within each group) using the 95% confidence interval (CI) results.

The Corrected Akaike Information Criterion (AIC_c) (Motulsky and Christopoulos, 2004) was calculated to select the most-likely-correct model, with the lower AIC_c value indicating the more-likely-correct model:

$$AIC_c = n \cdot ln\left(\frac{SS}{n}\right) + 2K + \frac{2K(K+1)}{n-K-1} \tag{16}$$

where n is the number of data points, SS is the sum of squares of residuals, and K is the number of parameters plus 1. The relative probability of each model being the correct model also was calculated as follows (Motulsky and Christopoulos, 2004):

$$\text{Relative likelihood of log} - \text{linear over Weibull model} = \frac{e^{\left(\frac{AIC_{c,log-linear\ model} - AIC_{c,Weibull\ model}}{2}\right)}}{1+e^{\left(\frac{AIC_{c,log-linear\ model} - AIC_{c,Weibull\ model}}{2}\right)}} \quad (17)$$

4.2 Results and Discussion

4.2.1 Water activity and moisture content of stored almonds

After 6 weeks of storage, the a_w of some of the group I replicates (Table 4.1) were lower than the initial range (0.45 ± 0.02 a_w), giving an average a_w of 0.43 a_w ($P < 0.05$); therefore, the samples were re-equilibrated at 45% RH for 5-7 days, and the a_w was measured again before performing any further thermal treatments. All of the group I sample replicates were re-equilibrated (0.45 ±0.02 a_w) prior to running any thermal treatments.

In contrast, the group II samples at 70 weeks were in the target a_w range (0.45 ±0.02) and were not different ($P > 0.05$) from the initial a_w value (week 0). At week 103, the a_w of the almonds (~0.471 a_w) was higher ($P < 0.05$) than week 0 (~0.452 a_w), but not significantly different ($P > 0.05$) to week 70 (~0.460 a_w). Therefore, the stored almonds from week 103 were thermally treated without re-equilibration, similar to the week 70 samples.

Table 4.1 The a_w (± standard deviation), and *Salmonella* Enteritidis PT 30 survival (± standard deviation) for whole almonds after 0 (groups I and II), 7 (I), 15 (I), 27 (I), 68 (I), 70 (II) and 103 (II) weeks of storage at room temperature (and prior to re-equilibration).

Storage time (weeks)	a_w	*Salmonella* survival (log CFU/g)
0 (I and II)	0.452 ± 0.005 ^{A}	8.5 ± 0.2 ^{A}
7 (I)	0.428 ± 0.002 ^{B}	8.5 ± 0.2 ^{A}
15 (I)	0.417 ± 0.001 ^{B}	8.5 ± 0.1 ^{A}
27 (I)	0.417 ± 0.003 ^{B}	7.8 ± 0.2 ^{B}
68 (I)	0.463 ± 0.003 ^{A}	6.2 ± 0.3 ^{C}
70 (II)	0.460 ± 0.002 $^{A, B}$	7.3 ± 0.1 ^{B}
103 (II)	0.471 ± 0.002 ^{B}	6.2 ± 0.3 ^{C}

Within a column (and within the same group), means with same superscript were not significantly different ($\alpha = 0.05$).

Although the a_w of the stored samples did change significantly in a few cases in this study, the changes were relatively small (< 0.04 a_w). In contrast, prior studies involving unsealed and sealed storage reported a_w changes of 0.30 (Zhang et al., 2017) to 0.40 a_w (Keller et al., 2013) and 0.20 a_w (Kimber et al., 2012), respectively. The type of containers and storage conditions (sealed or unsealed) clearly impacted a_w changes during storage.

In terms of moisture content, the two storage groups were not significantly different ($P >$ 0.05) at week 68(I) and 70(II) (3.9 and 3.8% MC, respectively), and the re-equilibration process did not have an impact on the moisture content of the almonds. Brar et al. (2015) reported that the moisture content of raw peanuts and pecan kernels remained stable after 52 weeks of storage at 22°C in sealed containers, whereas Kimber et al. (2012) reported a slight change in moisture

content during 28 weeks of storage (±1% MC). As expected, the moisture content of the stored group II almonds at week 70 and 103 was also stable at 3.8% ($P > 0.05$).

4.2.2 Survival of Salmonella Enteritidis PT 30 after storage at room temperature

For the group I samples, *Salmonella* Enteritidis PT 30 populations (Table 4.1) were stable until 15 weeks of storage, but then decreased after 27, and 68 weeks of storage. For the group II samples, the *Salmonella* Enteritidis PT 30 populations also decreased ($P < 0.05$) after 70 and 103 weeks of storage. *Salmonella* Enteritidis PT 30 populations were higher for the group II samples at 70 weeks of storage ($P < 0.05$) than for group I samples at 68 weeks of storage.

After 68 weeks of storage, the *Salmonella* Enteritidis PT 30 populations in the group I samples decreased 2.3 log CFU/g from initial counts, and at 70 weeks the group II samples decreased by 1.2 log CFU/g. In previous studies, the reduction of *Salmonella* Enteritidis PT 30 on almond kernels in sealed plastic bags (primary) and plastic tubs (secondary) (Abd et al., 2012) and sealed plastic bags (Kimber et al., 2012) were similar at 48 (2.1 log CFU/g; 23°C) and 50 weeks (2.3 log CFU/g; 24°C), respectively. However, Uesugi et al. (2006) reported a 3.4 log CFU/g reduction of *Salmonella* Enteritidis PT 30 on almond kernels after 68 weeks of storage at 23°C in sealed plastic bags, which was greater than for group I in the present study at 68 weeks (2.3 log CFU/g reduction) and group II at 70 weeks (1.2 log CFU/g reduction), which were in sealed tin cans. In addition, Brar et al. (2015) reported that *Salmonella* cocktail populations on pecans decreased by 0.4 log CFU/g after 10 weeks of storage in sealed plastic bags (22°C) and in another study were 1 log reduction lower after 10 weeks of storage in controlled glass or plastic desiccator jars (0.57 a_w; 25°C) (Santillana-Farakos et al., 2017). These results indicate that storage conditions

and container types are factors that likely impact *Salmonella* survival during long-term storage (Abd et al., 2012; Brar et al., 2015; Kimber et al., 2012).

In addition, homogeneity of the *Salmonella* Enteritidis PT 30 population after long-term storage was tested at week 74. Ten almonds from each replicate (30 samples in total per group) were randomly pulled from both groups. The mean populations for the group I (5.8 ± 0.7, 6.4 ± 0.8, and 5.6 ±1.0 log CFU/g; means ± SD of three replicates) were lower ($P < 0.05$) than in the group II samples (6.1 ± 0.9, 6.8 ± 0.3, 7.0 ± 0.2 log CFU/g). The environmental condition of group I was modified several times during the re-equilibration process, leading to a difference in a_w values between the two groups which may have affected *Salmonella* survival (Finn et al., 2013).

4.2.3 Reduction of Salmonella Enteritidis PT 30 during thermal come-up

Salmonella Enteritidis PT 30 populations decreased ($P < 0.05$) after thermal come-up at week 0, 15, and 103 (Figure 4.1); however, the reduction of *Salmonella* populations during thermal come-up did not change ($P > 0.05$) with increasing storage time (0.9 ± 0.4 log CFU/g).

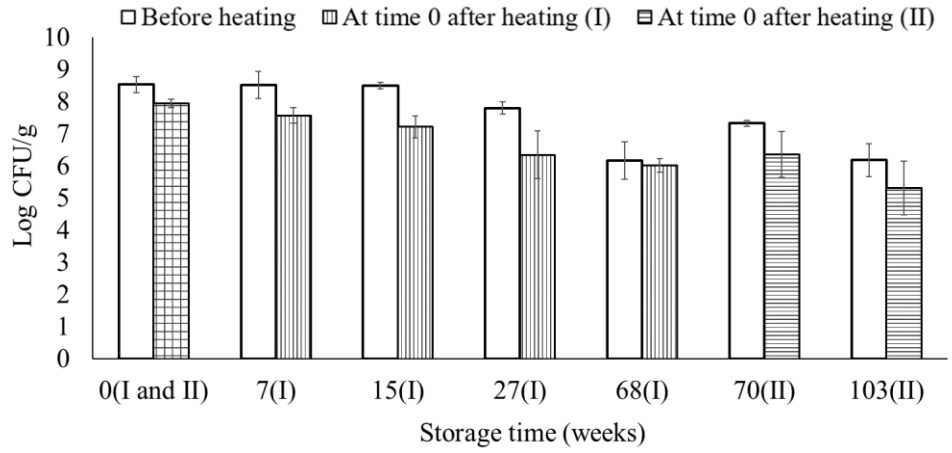

Figure 4.1 Survival (log CFU/g) of *Salmonella* Enteritidis PT 30 (mean values of triplicates ± standard deviation) on whole almonds (~0.45 a_w) after 0 (I and II), 7 (I), 15 (I), 27 (I), 68 (I), 70 (II) and 103 (II) weeks of storage at room temperature, and after reaching the come-up temperature in thermal inactivation trial (~80°C).

4.2.4 Thermal resistance of Salmonella Enteritidis PT 30 heated at 80°C

Salmonella Enteritidis PT 30 inactivation data (Figure 4.2) were used to estimate parameters of the log-linear and Weibull models. Results (Table 4.2) indicate that the log-linear model was the more-likely-correct model for 5 out of 7 data sets, but the relative likelihood was fairly low (54 – 81%); therefore, both models are presented.

However, the shape factor (p-value) at week 27 and 103 were not significantly different ($P > 0.05$) than zero, indicating the Weibull model was not a good choice in these cases.

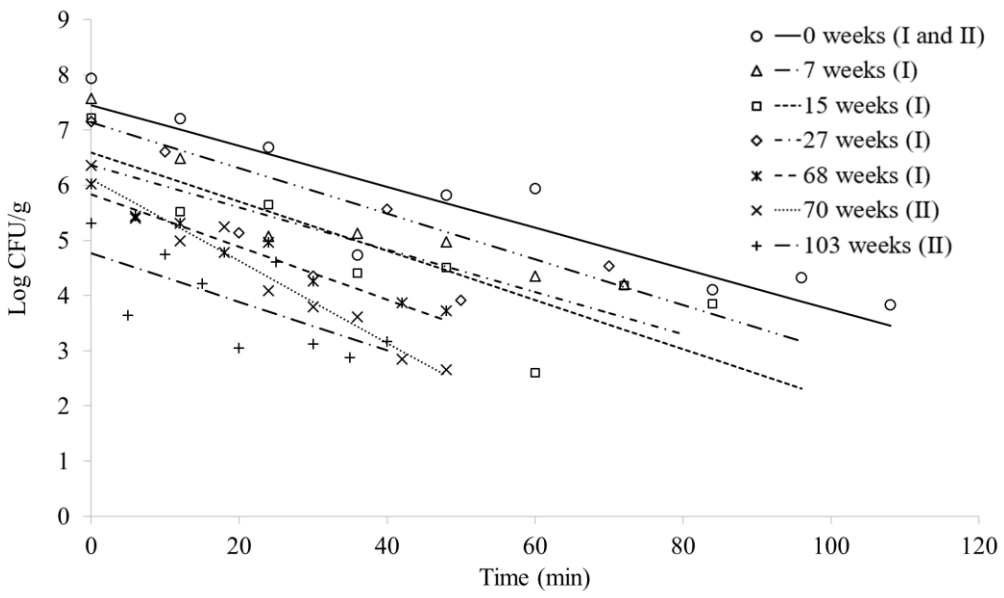

Figure 4.2 The survival (log CFU/g) of *Salmonella* Enteritidis PT 30 (mean values of triplicates and log-linear model) during isothermal heating (~80°C) of whole almonds (~0.45 a_w) after 0 (I and II), 7 (I), 15 (I), 27 (I), 68 (I), 70 (II) and 103 (II) weeks of storage at room temperature.

When the slope of the *Salmonella* Enteritidis PT 30 survival data (Figure 4.2) was compared using ANCOVA within the same group, thermal resistance of *Salmonella* Enteritidis PT 30 did not change for the group I samples during the entire storage period ($P > 0.05$). In the group II samples, thermal resistance of *Salmonella* Enteritidis PT 30 was lower ($P < 0.05$) in 70 week as compared to 0 week samples, but the 103 week samples were not different ($P > 0.05$) compared to the 70 week samples. It should be noted that the raw *Salmonella* populations data were determined from single kernels, which affects variability. The variances of group II individual kernel population data at 74 weeks were 0.2 to 0.9 log CFU/g, and the standard error of $D_{80°C}$ at 103 weeks was ± 8.4 min (37.1% of $D_{80°C}$).

Table 4.2 The $D_{80^{\circ}C}$, and δ and p Weibull parameter values (± standard errors) from the *Salmonella* Enteritidis PT 30 survivor curves for whole almonds (~0.45 a_w) after 0 (groups I and II), 7 (I), 15 (I), 27 (I), 68 (I), 70 (II) and 103 (II) weeks storage at room temperature.

Storage time (weeks)	Log-linear model			Weibull model				Relative likelihood of log-linear over Weibull model (per AIC_c)
	D-value* (min)	RMSE (log CFU/g)	AIC_c	δ* (min)	p*	RMSE (log CFU/g)	AIC_c	
0 (I and II)	27.0 ± 4.0 ^{A}	0.77	-4.9	9.9 ± 7.2 ^{A}	0.60 ± 0.17 ^{A}	0.72	-5.8	0.39
7 (I)	24.2 ± 4.2 ^{A}	0.58	-14.3	11.8 ± 8.0 ^{A}	0.59 ± 0.22 ^{B}	0.55	-14.0	0.54
15 (I)	22.4 ± 4.0 ^{A}	0.71	-4.9	5.3 ± 4.6 ^{A}	0.48 ± 0.15 $^{A, B}$	0.59	-8.7	0.13
27 (I)	26.1 ± 9.7 ^{A}	0.89	2.5	4.9 ± 10.1 ^{A}	0.44 ± 0.30 $^{A, B, **}$	0.85	3.8	0.66
68 (I)	20.9 ± 3.3 ^{A}	0.38	-21.2	15.0 ± 6.2 ^{A}	0.71 ± 0.23 $^{A, B}$	0.37	-18.8	0.77
70 (II)	13.5 ± 2.2 ^{B}	0.76	-6.0	10.7 ± 6.2 ^{A}	0.85 ± 0.30 ^{A}	0.78	-3.2	0.81
103 (II)	22.6 ± 8.4 $^{A, B}$	0.92	1.4	5.8 ± 11.4 ^{A}	0.35 ± 0.30 $^{A, **}$	0.92	3.3	0.72

* Within a column (and within the same group), means with common superscript letters were not significantly different (α = 0.05).

** This value is not significantly different (α = 0.05) from zero.

When comparing the two groups, the thermal resistance of *Salmonella* Enteritidis PT 30 was higher ($P < 0.05$) for group I samples at 68 weeks compared to group II samples at 70 weeks.

In prior studies, Abd et al. (2012) reported that the thermal resistance of *Salmonella* Enteritidis PT 30 during oil roasting of almonds (121°C) did not change after 48 weeks of storage at 23°C . While the relative humidity during storage was <40%, the moisture content and water activity of the samples were not monitored. However, the thermal resistance of *Salmonella* Enteritidis PT 30 on almonds after long-term storage at room temperature remained unchaged overall ($P > 0.05$) in both studies.

4.3 Conclusion

This study suggests that re-equilibrating almonds (group I) multiple times may have increased the rate of reduction of *Salmonella* populations during long-term storage. Overall, the findings support the hypothesis that thermal resistance of *Salmonella* on almonds does not change during storage, even after approximately two years. These results indicate that the validation of thermal pasteurization processes for almonds should not be affected by storage age of the almonds subjected to the process, which is important information for commercial operations.

5 EFFECTS OF PRODUCT STRUCTURE, TEMPERATURE, AND WATER ACTIVITY ON THE THERMAL RESISTANCE OF *SALMONELLA* ENTERITIDIS PT 30

Factors that have an impact on *Salmonella* thermal resistance in low-moisture foods, such as temperature and a_w, have never been compared for multiple product structures within the same type of product, such as almond meal and almond butter. In order to account for the effects that product structure, temperature, and a_w have on *Salmonella* thermal inactivation, multiple primary and secondary models were fit to inactivation data from almond, date, and wheat products.

5.1 Materials and Methods

The experimental design consisted of almond, date, and wheat products that were inoculated with *Salmonella*, fabricated into different structural forms after equilibration to 0.25, 0.45, and 0.65 a_w, and isothermally processed at three temperatures between 70-90°C. *Salmonella* thermal inactivation models then were developed from the data.

5.1.1 Wheat products

Organic soft white whole wheat kernels (*Triticum aestivum*, Eden Foods Inc., Clinton, MI) were stored in paper bags at room temperature (~20°C) for up to a year. Wheat meal and wheat flour were also produced from these wheat kernels after inoculation (See inoculation below) and equilibration (See equilibration below) to 0.25, 0.45, and 0.65 a_w. The wheat meal and flour products were produced from the inoculated and equilibrated kernels using a coffee grinder (model BCG1110B, KitchenAid, Benton Harbor, MI) inside an equilibration chamber (describe below) at the corresponding a_w setpoint, in order to prevent a_w changes during grinding. Wheat meal was produced by grinding the wheat kernels (50 g) for 25 s, with a pause every 10 s, to limit increases in product temperature. Wheat flour was produced using the same method as for wheat meal, but

was processed for 60 s of total time instead of 25 s. The size distribution for wheat meal and wheat flour were analyzed by using the American Society of Agricultural and Biological Engineers (ASABE) standard S319.2 – method of determining and expressing fineness of feed materials by sieving (Appendix C; Table C.1).

5.1.2 Almond products

Almonds (Nonpareil almonds, size 27/30) were sourced from a wholesale distributor (Select Harvest, Turlock, CA), vacuum-packed (350 g/bag) and stored at ~2.5°C for up to two years. Prior to fabricating almond meal and butter, the almond kernels were inoculated (See inoculation below) and equilibrated (See equilibration below). Once the almond kernels were at equilibrium (0.25, 0.45, or 0.65 a_w), almond meal and butter were fabricated in a food processor (model FP21, Hamilton Beach Brands, Inc., Glen Allen, VA) that was also placed in an equilibration chamber to prevent a change in a_w after fabrication. The almond kernels (100 g) were processed for 45 s at the lowest speed setting into almond meal. For almond butter, 200 g of almond kernels were ground similarly, but in 2 min time intervals, for a total of 16 min. Dry ice (~30 ml) was added every 2 min to maintain the product temperature below 40°C, which was monitored with a handheld infrared thermometer (Fluke IR 566, Everett, Washington). The size distribution of almond meal (Appendix C; Table C.2) was analyzed by Microtrac Laser light scattering (model S3500, Micotrac Inc, Montgomeryville, PA).

5.1.3 Date products

Dates (Medjool, jumbo) were purchased from a retail supplier (Nuts.com, Cranford, NJ) and stored in plastic bags at ~2.5°C for up to two years. The whole dates were cut into pieces (10

x 10 x 0.5 mm), each of which consisted of a 10 x 10 mm piece of date skin, which were called date pieces. To produce the date paste, whole dates were pitted, cut into smaller chunks, inoculated (See inoculation below), and equilibrated (See equilibration below) before processing three consecutive times through a meat grinder with holes 1 cm in diameter (KitchenAid, model K5-A, Benton Harbor, MI) to ensure date paste homogeneity.

5.1.4 Inoculation

The inoculation procedures of Danyluk et al. (2005) were used to almond and wheat products. *Salmonella enterica* serovar Enteritidis PT 30, previously obtained from Dr. Linda Harris (University of California, Davis), was kept frozen (-80°C) as a concentrated culture in Trypticase Soy Broth (TSB; Difco, BD, Franklin Lakes, NJ) containing 20% (vol/vol) glycerol. One loopful (10 µl) of frozen culture was subjected to two successive 24 h (37ºC) transfers in 10 ml of 17% (m/m) TSB containing 0.6% yeast extract (Difco, BD) (TSBYE). Thereafter, a 150 by 15 mm plate of Trypticase Soy Agar (TSA; Difco, BD) containing 0.6% yeast extract (TSAYE) was spread with 1 ml of inoculum to obtain confluent growth after 24 h incubation (37°C).

For wheat and almond products, the *Salmonella* lawn culture from the TSAYE plate was harvested in 10 ml of 0.1% peptone water using a L-shaped spreader. The resulting 8 ml *Salmonella* suspension (~$10^{7.5}$ to 10^{9} CFU/ml) was added directly to 100 g of either wheat or almond kernels and mixed in a sterile plastic bag by hand for 1 min, placed on filter paper (P8, Fisher Scientific, Pittsburgh, PA) in an open plastic container, dried (~3 h) in a biosafety cabinet, and then placed in an equilibration chamber (See equilibration below). After reaching their prescribed target a_w, the inoculated wheat and almond products were processed as described in their respective material sections (See almond products and wheat products above).

The date products were inoculated differently due to their size. As described by Danyluk et al. (2005), the *Salmonella* was grown in lawn plates; however, rather than 10 ml, 20 ml of 0.1% peptone water was used for harvesting with a L-shape spreader, after which the cell suspension was centrifuged (model Sorvall RC 6 plus, SS-34 rotor, Thermo Fisher Scientific, MA) at 2,988 × *g* for 15 min. The resulting *Salmonella* pellet was resuspended in 2 ml of 0.1% peptone water and subsequently homogenized using a vortex mixer (model G-560, Scientific Industries Inc., Bohemia, NY), which yielded a liquid suspension containing ~10^{11} CFU/ml. For the date pieces, 50 µl was pipetted onto the outer skin of each piece, dried (≥ 20 min) in a biosafety cabinet, and then placed into an equilibration chamber.

Production of the date paste began by cutting whole dates into 12 equally sized pieces (~1.8 g each) for more homogenous equilibration. Each date piece was spot inoculated (200 µl total inoculum across 12 pieces) on the outer skin, dried for ≥ 20 min in a biosafety cabinet, and then placed in an equilibration chamber before grinding as described in the material section (See date products above).

5.1.5 Equilibration

The target water activities for all samples were 0.25±0.02, 0.45±0.02, and 0.65±0.02 a_w. Custom-designed equilibration chambers (Smith and Marks, 2015) were used to modify and control the a_w of all samples. Relative humidity in the chambers was monitored and controlled by a humidity sensor (DHT 22, Adafruit Industries, New York, NY) and a microcomputer (Mega 2560, Arduino, Italy), with humidity-controlled air (±0.2% R.H. of chamber setpoint) obtained by circulating air through a desiccant (dry air) or water column (moist air). All of the samples, except for almond butter, were spread in a thin (< 5 mm) or single layer on open-mesh metal trays (almond

kernels; Appendix D; Figure D.1) or filter paper trays (wheat kernels, wheat meal, wheat flour, almond meal, date pieces and date paste; date paste was shaped into a 15 mm diameter sphere to reduce equilibration time). The almond butter was placed into a 16 oz. tin can and continuously stirred with a stainless steel rod, controlled by a motor (Mini 12 V., 60 rpm, high torque gear box electric motor, Nextrox, Newark, DE) (Figure D.2), to mitigate oil-water separation in the butter. Equilibration times for each of the sample types were dependent on their respective adsorption/desorption characteristics (Table 5.1).

Table 5.1 Equilibration time for wheat, almond, and date products.

Product	**Equilibration time**
Wheat kernels	5-7 days
Wheat meal	1-3 days after fabrication
Wheat flour	1-3 days after fabrication
Almond kernels	5-7 days
Almond meal	1-3 days after fabrication
Almond butter	5-7 days after fabrication
Date pieces	5-7 days
Date paste	5-7 days after fabrication

5.1.6 Water activity measurement

The a_w of all samples was measured using a a_w meter (AquaLab 4TE, Decagon Devices, Pullman, WA) to confirm that the target a_w was achieved.

5.1.7 Water activity measurement at 80°C

The samples were equilibrated until they reached the target a_w at 25°C (0.25, 0.45, and 0.65 a_w), confirmed via the a_w meter before heating. The equilibrated samples were then placed in a custom-designed high temperature a_w meter (Figure D.3; Decagon Devices, Pullman, WA), which was placed in a hot air oven (model 725F, Thermo Fisher Scientific, MA) that was set at 83°C. The a_w and temperature were recorded every 10 s. Once the sample temperature reached 79.5°C, the samples were held for 10 min (T < 80.5°C) in the oven, and the average a_w value from the last 2 min was calculated (the change in a_w value < 0.01 during the 10 min holding time). The a_w values reported at 25°C and 80°C were averages from duplicate experimental trials.

5.1.8 Differential scanning calorimetry

Wheat flour, almond butter, and date paste were equilibrated at 0.25, 0.45, 0.65 a_w (25°C). After equilibration, the samples (10 mg for wheat flour, 15 mg for almond butter, and 20 mg for date paste) were placed in a sealed aluminum pan and heated at 0.5°C/min from 20°C to 100°C in a differential scanning calorimeter (DSC) (model 2000, TA instruments, New Castle, DE). The glass transition temperature (T_g) was assigned an inflection point based upon the transition temperature span. The characteristic temperature (peak temperature, T_p) and total heat of transition (ΔH) were determined for the peak temperature and the area under the heating curve, where

characteristic transition occurred. Samples were measured twice, and the results were calculated using Universal Analysis 2000 software (TA instruments).

5.1.9 Thermal treatment

After equilibration to the target a_w, almond kernels (1 kernel, ~1.2 g), wheat kernels (7 kernels, ~0.4 g), and date pieces (1 piece, ~0.9 g) were vacuum sealed in a single layer (< 1 mm) in plastic bags (4 oz., Nasco, Fort Atkinson, WI). The fabricated products (0.7 g of almond meal, 1.2 g of almond butter, 0.6 g of wheat meal, 0.5 g of wheat flour, and 1.2 g of date paste) were loaded into aluminum test cells (sample thickness < 1 mm) and then sealed (Chung et al., 2008). All of the sample containers were packed inside the equilibration chambers to prevent any change in a_w, which could occur if packaged in the non-humidity-controlled laboratory environment.

For all isothermal treatments (Table 5.2), water baths (GP-400, Neslab, Newington, NH) were set 0.5°C above the target temperature (70, 75, and 80°C for date products, 80, 85, and 90°C for almond and wheat products; Table 5.2). The come-up time was established by immersing a sample into the water bath and removing it when the temperature was 0.5°C below the target temperature. Come-up times for each product type were computed from the average of six replicate samples at each of the target temperatures (See Appendix E). After the samples had reached the come-up time, the initial (time zero) sample was removed and immediately cooled in an ice bath. The remaining samples for each trial were removed at pre-determined time points (9 points total for each trial) and cooled prior to microbial analysis.

Table 5.2 Experimental design for the thermal inactivation of *Salmonella* Enteritidis PT30 on almond, wheat, and date products at 0.25, 0.45, and 0.65 a_w between 70-90°C.

Sample		Low Temperature			Medium Temperature			High Temperature		
		0.25 a_w	0.45 a_w	0.65 a_w	0.25 a_w	0.45 a_w	0.65 a_w	0.25 a_w	0.45 a_w	0.65 a_w
Almond	Kernels	80°C			85°C			90°C		
	Meal									
	Butter									
Wheat	Kernels	80°C			85°C			90°C		
	Meal									
	Flour									
Date	Pieces	70°C			75°C			80°C		
	Paste									

5.1.10 Recovery and enumeration

After cooling, the samples were aseptically removed from their containers, before being diluted 1:10 dilution in 0.1% peptone water and homogenized in a stomacher for 3 min (Model 1381/471, NEU-TEC Group Inc., Farmingdale, NY). From the initial dilution, multiple serial dilutions were prepared and dispensed onto mTSAYE (TSAYE supplemented with 0.05% of ammonium ferric citrate and 0.03% of sodium thiosulfate pentahydrate; Fisher Chemical, Fair Lawn, NJ) in duplicate. The plates were incubated for 48 h at 37°C prior to counting the *Salmonella*

colonies, which appeared black on this non-selective, differential medium. Testing of uninoculated samples with this medium revealed no *Salmonella*-like colonies (< 2 log CFU/g).

5.1.11 Statistical analyses of properties

Water activity values at 25 and 80°C were compared using Analysis of Variance (ANOVA) with Tukey's test (Minitab 17 Statistical Software, Minitab, Inc., State College, PA). The DSC parameters were also compared via ANOVA with Tukey's test (Minitab).

5.1.12 Generalized linear model for testing factors affecting Salmonella inactivation

The effects of product structure, temperature, and a_w on the *Salmonella* inactivation data (Appendix F) were analyzed using the generalized linear model via MATLAB (version R2017b, MathWorks Inc., Natick, MA; Appendix G). Time, product structure, temperature, and a_w were all evaluated. The main effects, two-way interactions, and three-way interactions of these variables were included in the model.

$$\begin{aligned}\log N = {} & \beta_0 + \beta_1 \times t + \beta_2 \times T + \beta_3 \times a_w + \beta_4 \times S + \beta_5 \times t \times T + \beta_6 \times t \times a_w + \beta_7 \times t \times S + \\ & \beta_8 \times T \times a_w + \beta_9 \times T \times S + \beta_{10} \times a_w \times S + \beta_{11} \times t \times T \times a_w + \beta_{12} \times t \times T \times S + \\ & \beta_{13} \times t \times a_w \times S + \beta_{14} \times T \times a_w \times S \end{aligned} \quad (18)$$

where N is the population (CFU/g) at t, T, a_w, and S, t is the time of the isothermal treatment (min), T is the temperature of the isothermal treatment (°C), a_w is the initial a_w of the sample, and S is the product structure of sample.

5.1.13 Primary models

Salmonella survival data (triplicates) within each treatment were used to estimate the log-linear and Weibull parameters using nlinfit (nonlinear regression routine in the statistical toolbox) in MATLAB (Appendix H). The log-linear model was estimated by the following equation:

$$\log N = -\frac{t}{D(T)} + \log N_0 \quad (19)$$

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), and $D(T)$ is the time (min) required to reduce the microbial populations by 90% at a specified temperature (T, °C).

The Weibull model parameters were estimated, according to the following equation (Peleg, 2006):

$$\log \mathrm{N} = -\left(\frac{t}{\delta}\right)^p + \log N_0 \quad (20)$$

where p is the shape factor, and δ is the location factor (min).

5.1.14 Secondary model

A preferred secondary model was developed after evaluating the primary models by the root mean square error (RMSE) and Corrected Akaike Information Criterion (AIC_c) (See model performance and selection below). In this study, product structure was not applied within the secondary model because it was a discrete class variable, unlike the continuous variables of temperature and a_w.

The *Salmonella* survivor date (log N/N_0) were used to estimate parameters for a Bigelow-type model (Gaillard et al., 1998) with modifications to account for the effects of temperature and a_w on the D-value:

$$\log D_{T,a_w} = \log D_{ref} - \left(\frac{T - T_{ref}}{z_T}\right) - \left(\frac{a_w - a_{w,ref}}{z_{a_w}}\right) \quad (21)$$

where D_{ref} is the time required to reduce the microbial populations by 90% (1 log reduction) at $T = T_{ref}$ and $a_w = a_{w,ref}$; T is the temperature (°C); T_{ref} is the optimized reference temperature (°C); $a_{w,ref}$ is the optimized reference for a_w (a_w is between 0 to 1); Z_T and Z_{aw} are the temperature (°C) and a_w changes required to increase or decrease the D-value by 1 log.

The reference temperature (T_{ref}) and reference a_w ($a_{w,ref}$) were optimized to minimize the correlation between parameters for the smallest relative errors of estimated parameters (Dolan et al., 2013). To estimate parameters in the secondary models, the reference a_w was held constant while the reference temperature was varied between a minimum and maximum process temperature. The correlation coefficient between D_{ref}, and Z_T, was plotted, which yielded an optimized reference temperature at the value with the smallest correlation coefficient. Next, the T_{ref} was held constant at the optimized value, and the procedure above was repeated to determine an optimized $a_{w,ref}$. This two-step optimization procedure for T_{ref} and $a_{w,ref}$ was iterated two additional times to yield the final optimized reference conditions.

5.1.15 Model performance and selection

Model performance was evaluated based on RMSE (Motulsky and Christopoulos, 2004), the AIC_c (Motulsky and Christopoulos, 2004), and the scaled sensitivity coefficient (SSC) (Dolan

and Mishra, 2013). Additionally, the estimated parameters were evaluated using the 95% confidence intervals (CI) and their relative errors.

The RMSE was calculated using the following equation (Motulsky and Christopoulos, 2004):

$$RMSE = \sqrt{\frac{\sum\left(\log N_{predicted} - \log N_{observed}\right)^2}{n-m}} \quad (22)$$

where $N_{predicted}$ and $N_{observed}$ are the predicted and observed *Salmonella* populations (CFU/g) at each time; n is the number of observation points; and m is the number of model parameters.

The AIC_c was used to select the most-likely-correct model for primary and secondary model evaluation. A lower AIC_c value indicates the more-likely-correct model:

$$AIC_c = n \cdot ln\left(\frac{SS}{n}\right) + 2K + \frac{2K(K+1)}{n-K-1} \quad (23)$$

where n is the number of data points; SS is the sum of squares of residuals, and K is the number of parameters plus 1. The relative likelihood was also calculated for each treatment for selection of the correct model (Motulsky and Christopoulos, 2004):

$$\text{Relative likelihood of loglinear over Weibull model} = \frac{e^{\left(\frac{AIC_{c,log-linear\ model} - AIC_{c,Weibull\ model}}{2}\right)}}{1+e^{\left(\frac{AIC_{c,log-linear\ model} - AIC_{c,Weibull\ model}}{2}\right)}} \quad (24)$$

SSC was calculated to test the correlation between model parameters and the unique estimability of each parameter. Large SSCs indicated low correlation of parameters (Beck and Arnold, 1997):

$$X'_{\beta_i} = \beta_i \times \frac{\partial f(t,\beta_i)}{\partial \beta_i} \quad (25)$$

For the secondary models, simulated temperature and a_w profiles (Figure 5.1) were generated to represent arbitrary and varying experimental conditions across the range of temperature and a_w values for all experiments (which were isothermal and iso-a_w); however, the estimated parameter cannot be calculated without variance. Therefore, temperature and a_w (increasing and decreasing, respectively) were used to determine the variance of the simulated experiments. The temperature profile increased linearly from 70 to 80°C for date products, and from 80 to 90°C for almond and wheat products. Similarly, the a_w profile decreased linearly from 0.65 to 0.25.

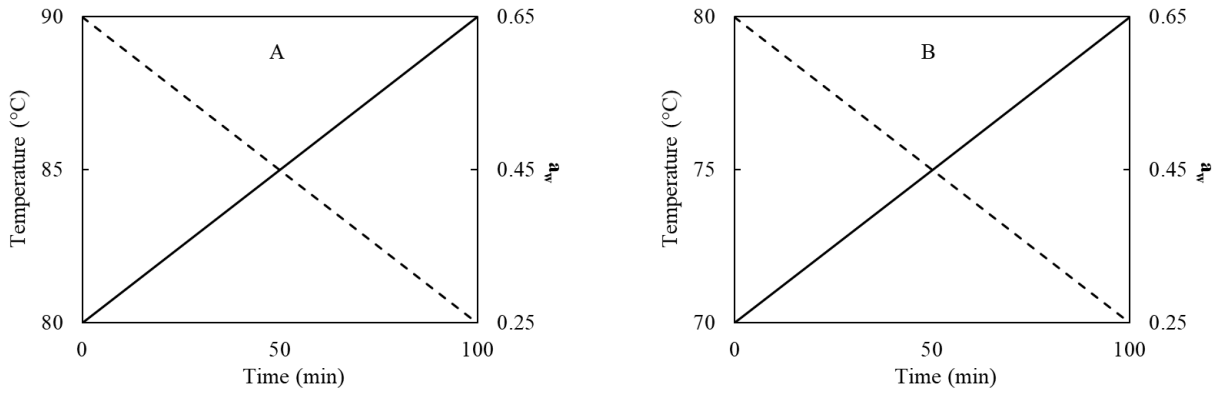

Figure 5.1 Simulated temperature and a_w profiles for: (A) almond and wheat products, and (B) date products. Solid line is simulated temperature, and dashed line is simulated a_w.

5.2 Results and discussion

5.2.1 Initial inoculation

Homogeneity of the *Salmonella* populations were calculated for each product structure and type across all temperatures and a_w value. The mean (± standard deviation) initial *Salmonella* populations for inoculated wheat kernels, meal, and flour were 8.94 ± 0.23, 8.56 ± 0.31, and 8.49 ± 0.18 log CFU/g, respectively. For almond kernels, meal, and butter, the initial *Salmonella* populations were 8.41 ± 0.24, 8.19 ± 0.18, and 8.25 ± 0.40 log CFU/g, respectively. For the date pieces and paste, the mean *Salmonella* populations were 9.04 ± 0.41 and 8.06 ± 0.34, respectively.

After reaching the come-up temperature at time 0, the *Salmonella* populations had decreased 0.02 – 3.41, 0.07 - 2.81, and 0.04 – 2.89 log CFU/g for wheat, almond, and date products, respectively (Appendix I). The greatest reduction occurred when the temperature and a_w were at their maximum values.

5.2.2 Generalized linear model (GLM)

The GLM was developed using the *Salmonella* inactivation data (Appendix F) to determine the impact of time, temperature, product structure, and a_w of wheat, almond, and date products on *Salmonella* survival, including the interaction between these parameters. Because samples were treated in sealed containers, temperature and a_w remained static and did not change within any single experiment.

Across all products, *Salmonella* populations decreased more rapidly when the temperature and a_w were increased (Figure 5.2 and Appendix F). In addition, products with similar structures (large particle – kernels and pieces), but difference compositions, resulted in significantly different

lethality (Figure 5.2C). The *Salmonella* lethality rate was faster for date pieces than for wheat kernels and almond kernels. In comparison, the lethality of *Salmonella* was lower in peanut butter than in wheat flour when samples were equilibrated to the same water activity (0.45 a_w) and thermally treated at 80°C (Syamaladevi et al., 2016a), which is consistent with the almond-wheat comparison in this study.

Within the same product type, product structure influenced the *Salmonella* inactivation results (Figure 5.3) for almond and date products. *Salmonella* lethality rates in fabricated products (i.e., meal, butter, and paste) were lower compared to whole products (non-fabrication). However, wheat product structure did not impact the lethality of *Salmonella* at 0.45 a_w and T = 80°C.

Based on the GLM regression (Table 5.3-5.5), the interactions of product structure with time, and the interaction of temperature and product structure with time, both had an effect on *Salmonella* inactivation in all products ($P < 0.05$). For a_w, the interaction of a_w and time had an effect ($P < 0.05$) on *Salmonella* inactivation in wheat and almond products, but was not significant ($P > 0.05$) for the date products. *Salmonella* inactivation in date products was impacted by the interaction of a_w and structure. Overall, all of these results indicate that temperature, product structure, and a_w impacted *Salmonella* inactivation for all of the products. However, to further understand the nature of the relation between *Salmonella* inactivation and product structure, temperature, and a_w for low-moisture foods, primary and secondary kinetic models were needed.

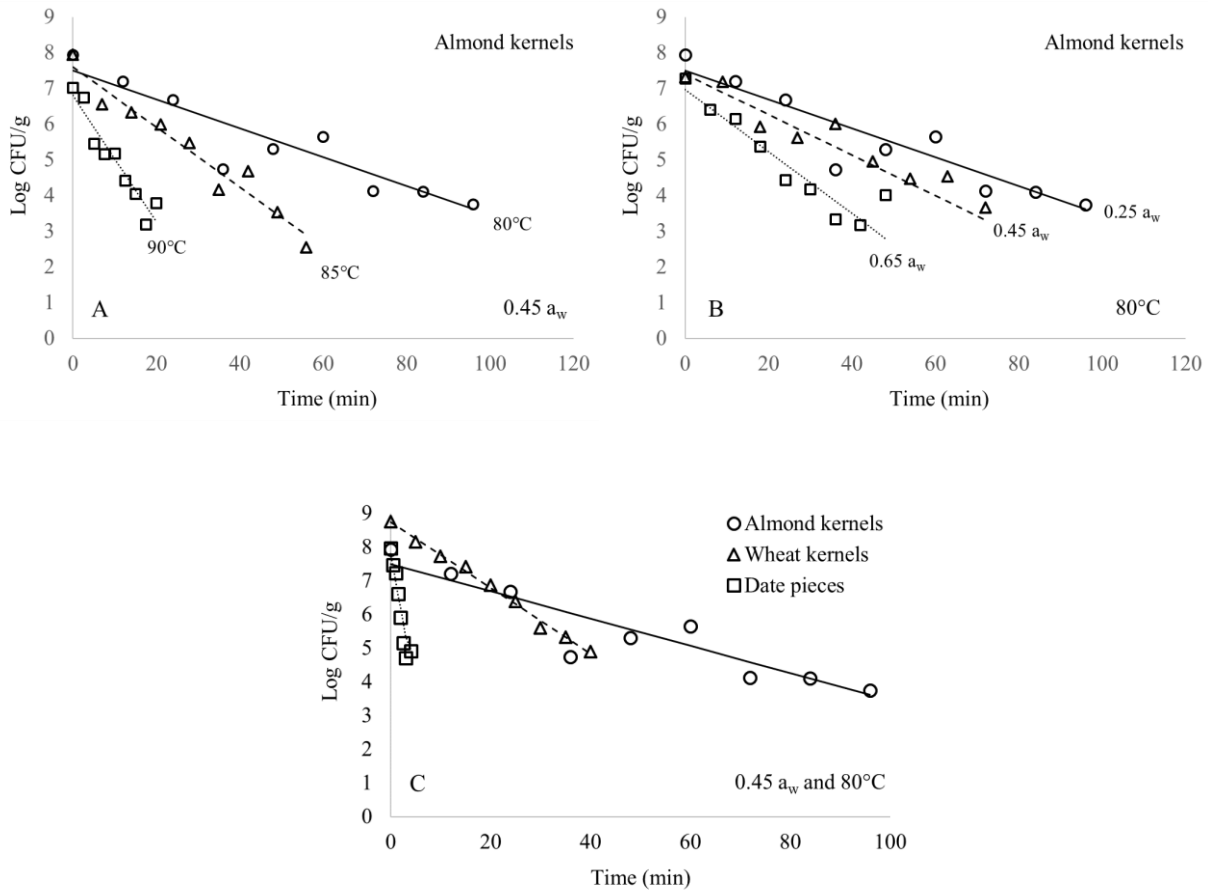

Figure 5.2 Isothermal (80°C) *Salmonella* survival curves and log-linear model fit for: (A) almond kernels at 0.45 a_w and three different temperatures (80, 85, and 90°C), (B) almond kernels at three different a_w (0.25, 0.45, and 0.65) and at 80°C, and (C) almond kernels, wheat kernels, and date pieces at 0.45 a_w and 80°C.

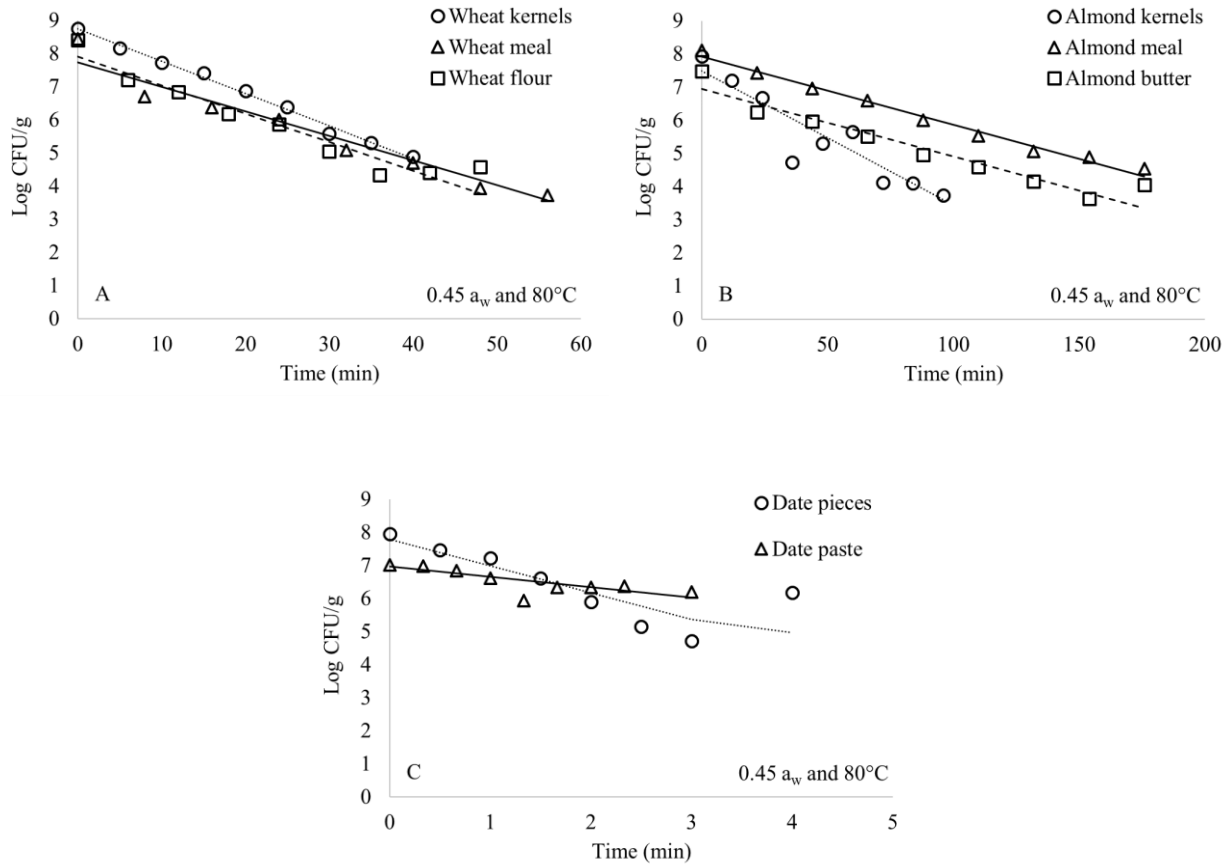

Figure 5.3 Isothermal (80°C) *Salmonella* survival curves and log-linear model fit for: (A) wheat products, (B) almond products, and (C) date products at 0.45 a_w.

Table 5.3 GLM regression for the effect of treatment on *Salmonella* inactivation (log CFU/g) in wheat products ($\alpha = 0.05$)

Source	Estimate	SE	tStat	P value
t	-1.22	0.29	-4.20	0.00*
T	-0.04	0.09	-0.45	0.65
a_w	6.08	15.37	0.40	0.69
Structure	3.86	3.57	1.08	0.28
t x T	0.02	0.00	4.42	0.00*
t x a_w	9.32	0.82	11.43	0.00*
t x structure	-0.26	0.07	-3.60	0.00*
T x a_w	-0.08	0.18	-0.45	0.65
T x structure	-0.05	0.04	-1.10	0.27
a_w x structure	1.40	6.97	0.20	0.84
t x T x a_w	-0.12	0.01	-12.04	0.00*
t x T x structure	0.00	0.00	3.83	0.00*
t x a_w x structure	0.01	0.03	0.40	0.69
T x a_w x structure	-0.03	0.08	-0.39	0.70

*Significant term at $\alpha = 0.05$.

Table 5.4 GLM regression for the effect of treatment on *Salmonella* inactivation (log CFU/g) in almond products ($\alpha = 0.05$)

Source	Estimate	SE	tStat	P value
t	-0.23	0.13	-1.76	0.08
T	-0.19	0.10	-1.94	0.05
a_w	-10.55	16.43	-0.64	0.52
Structure	-3.76	4.09	-0.92	0.36
t x T	0.00	0.00	1.75	0.08
t x a_w	0.64	0.22	2.92	0.00*
t x structure	0.12	0.04	2.80	0.01*
T x a_w	0.16	0.19	0.83	0.41
T x structure	0.07	0.05	1.42	0.16
a_w x structure	8.70	7.85	1.11	0.27
t x T x a_w	-0.01	0.00	-3.12	0.00*
t x T x structure	0.00	0.00	-2.85	0.00*
t x a_w x structure	-0.01	0.01	-1.02	0.31
T x a_w x structure	-0.14	0.09	-1.52	0.13

*Significant term at $\alpha = 0.05$.

Table 5.5 GLM regression for the effect of treatment on *Salmonella* inactivation (log CFU/g) in date products ($\alpha = 0.05$)

Source	Estimate	SE	tStat	P value
t	4.79	0.95	5.06	0.00*
T	-0.27	0.13	-2.04	0.04*
a_w	-38.25	20.61	-1.86	0.06
Structure	-16.11	6.22	-2.59	0.01*
t x T	-0.07	0.01	-5.42	0.00*
t x a_w	0.72	1.24	0.58	0.56
t x structure	-2.08	0.39	-5.30	0.00*
T x a_w	0.50	0.27	1.86	0.06
T x structure	0.21	0.08	2.64	0.01*
a_w x structure	44.81	12.84	3.49	0.00*
t x T x a_w	-0.01	0.02	-0.48	0.63
t x T x structure	0.03	0.01	5.81	0.00*
t x a_w x structure	-0.26	0.10	-2.58	0.01*
T x a_w x structure	-0.62	0.17	-3.70	0.00*

*Significant term at $\alpha = 0.05$.

5.2.3 Primary models

Primary model parameters were estimated for each *Salmonella* inactivation data set (triplicate) for every combination of temperature, product structure and type, and a_w for both the log-linear and Weibull models (Table 5.6-5.9). Results indicated that the log-linear model was the more-likely-correct model for wheat (17/27), almond (18/27), and date (16/18) products. However, the % likelihood was fairly low (51 – 80% for wheat products, 52 – 80% for almond products, and 61 – 86% for date products). Therefore, both parameters for both models were presented to compare *Salmonella* thermal resistance across all products (Table 5.6-5.9).

Few studies have compared the log-linear and the Weibull models for isothermal treatment of low-moisture products. Villa-Rojas et al. (2013) reported that the Weibull model better predicted *Salmonella* Enteritidis PT 30 inactivation in almond kernel flour. However, the primary models from Villa-Rojas et al. were analyzed using the R^2 value, which will always favor the Weibull model over the simpler log-linear model. In the present study, the log-linear model was more-likely-correct (AIC_c likelihood ~65%) for almond meal at 0.65 a_w and T = 80°C.

For thermal inactivation of *Salmonella* in wheat flour, Smith et al. (2016) suggested that the log-linear model was more-likely-correct for 75 – 85°C and a_w values of 0.310 – 0.700 based on AIC_c. The likelihood was ~66 - 76% for all a_w at T = 80°C, which was similar to wheat flour in this study, except for 0.25 a_w at 85 and 90°C.

Santillana-Farakos et al. (2013) reported that the Weibull model better fit the *Salmonella* inactivation data for whey protein powder (0.19 – 0.43 a_w, 21 – 80°C); however, their analyses also were based on adjusted R^2 and RMSE values. As noted above, these performance measures will always favor the Weibull over log-linear models, which is why AIC_c is an important comparison tool.

Based on the AIC_c results, the log-linear model generally was the more-likely-correct model, but not for all of the individual products. In developing the secondary model, the Weibull shape factor p was considered for each product type. The shape factor was between 0.60 – 1.69 for wheat products, 0.61 – 1.20 for almond products, and 0.61 – 1.43 for date products. Although, the relationship between shape factor and temperature/a_w (Appendix J) was tested, no statistically significantly relationship between shape factor and temperature was seen for any of the products. The relationship between a_w and shape factor was also random (i.e., no trend) for almond and date products. However, the shape factor did increase with increasing a_w in wheat products ($P < 0.05$).

In addition, the shape factor p was compared to p =1 using a paired t-test for all products. The shape factors from the date products were not significantly different ($P > 0.05$). For the wheat and date products, most of the shape factors were also not significantly different from 1 ($P > 0.05$; 23/27 correct for wheat products and 25/27 correct for almond products). Therefore, this analysis additionally supports the choice of the log-linear model, given the absence of any systematic trends in the shape factor.

Consequently, the log-linear model (p =1) was selected to further develop the secondary model in order to account for the effects of temperature and a_w for all products.

Table 5.6 Parameter estimates (mean ± standard error) for the log-linear and Weibull models, root mean squared errors (RMSE), and AIC_c values for wheat kernels, meal, and flour.

Products		Log-linear model			Weibull model				
		D-value (min)	RMSE (log CFU/g)	AIC_c	δ (min)	p	RMSE (log CFU/g)	AIC_c	Relative likelihood of log-linear over Weibull model (per AIC_c)
Wheat kernels									
0.25 a_w	80°C	20.1 ± 2.1	0.66	-17.8	21.1 ± 8.1	1.03 ± 0.27	0.67	-15.0	0.80
	85°C	10.3 ± 0.7	0.49	-33.4	8.2 ± 2.6	0.88 ± 0.14	0.49	-31.4	0.73
	90°C	4.7 ± 0.4	0.64	-16.9	3.0 ± 1.3	0.79 ± 0.16	0.63	-16.0	0.62
0.45 a_w	80°C	10.2 ± 1.0	0.61	-21.8	10.7 ± 3.8	1.03 ± 0.25	0.62	-19.0	0.80
	85°C	3.3 ± 0.2	0.44	-39.1	5.1 ± 0.8	1.38 ± 0.17	0.40	-43.0	0.13
	90°C	1.2 ± 0.1	0.45	-36.1	1.5 ± 0.3	1.16 ± 0.17	0.45	-34.7	0.67
0.65 a_w	80°C	3.7 ± 0.2	0.67	-17.0	6.2 ± 1.4	1.36 ± 0.21	0.63	-18.3	0.34
	85°C	1.4 ± 0.1	0.45	-36.5	1.7 ± 0.3	1.15 ± 0.14	0.45	-35.3	0.64
	90°C	0.5 ± 0.0	0.56	-26.4	0.4 ± 0.1	0.93 ± 0.15	0.57	-24.0	0.77

Parameters were estimated only in each a_w and temperature condition, and only compared within each row.

Table 5.6 Parameter estimates (mean ± standard error) for the log-linear and Weibull models, root mean squared errors (RMSE), and AIC_c values for wheat kernels, meal, and flour (cont'd).

Products		Log-linear model			Weibull model				
		D-value (min)	RMSE (log CFU/g)	AIC_c	δ (min)	p	RMSE (log CFU/g)	AIC_c	Relative likelihood of log-linear over Weibull model (per AIC_c)
Wheat meal									
0.25 a_w	80°C	33.5 ± 1.3	0.19	-83.5	21.9 ± 2.9	0.75 ± 0.05	0.15	-94.2	~0.00
	85°C	18.1 ± 1.5	0.46	-36.7	10.4 ± 3.8	0.72 ± 0.13	0.44	-37.7	0.38
	90°C	5.4 ± 0.3	0.36	-50.3	3.3 ± 0.8	0.76 ± 0.08	0.32	-54.4	0.11
0.45 a_w	80°C	13.5 ± 1.5	0.74	-10.2	4.5 ± 2.8	0.60 ± 0.13	0.68	-12.6	0.23
	85°C	4.3 ± 0.5	0.78	-8.1	2.0 ± 1.2	0.68 ± 0.16	0.75	-8.4	0.47
	90°C	1.0 ± 0.1	0.68	-14.1	0.8 ± 0.4	0.89 ± 0.24	0.69	-11.5	0.78
0.65 a_w	80°C	3.8 ± 0.3	0.50	-32.5	3.6 ± 1.1	0.96 ± 0.18	0.51	-29.8	0.80
	85°C	1.3 ± 0.1	0.35	-52.2	1.3 ± 0.3	1.01 ± 0.12	0.35	-49.4	0.80
	90°C	0.5 ± 0.1	0.78	-8.2	0.7 ± 0.2	1.69 ± 0.69	0.77	-7.2	0.63

Parameters were estimated only in each a_w and temperature condition, and only compared within each row.

Table 5.6 Parameter estimates (mean ± standard error) for the log-linear and Weibull models, root mean squared errors (RMSE), and AIC_c values for wheat kernels, meal, and flour (cont'd).

Products		Log-linear model			Weibull model				
		D-value (min)	RMSE (log CFU/g)	AIC_c	δ (min)	p	RMSE (log CFU/g)	AIC_c	Relative likelihood of log-linear over Weibull model (per AIC_c)
Wheat flour									
0.25 a_w	80°C	37.0 ± 4.1	0.61	-21.5	27.7 ± 12.8	0.83 ± 0.22	0.62	-19.3	0.75
	85°C	20.7 ± 1.6	0.47	-31.0	8.4 ± 3.0	0.64 ± 0.09	0.39	-38.7	0.02
	90°C	6.4 ± 0.5	0.64	-18.6	3.3 ± 1.5	0.72 ± 0.13	0.61	-19.4	0.40
0.45 a_w	80°C	11.6 ± 1.5	0.80	-6.2	5.0 ± 3.4	0.65 ± 0.18	0.77	-6.3	0.50
	85°C	3.5 ± 0.5	0.75	-10.8	2.2 ± 1.4	0.75 ± 0.24	0.75	-9.1	0.70
	90°C	0.9 ± 0.1	0.67	-16.0	0.9 ± 0.4	1.03 ± 0.34	0.68	-13.2	0.80
0.65 a_w	80°C	3.3 ± 0.2	0.36	-50.4	2.7 ± 0.6	0.89 ± 0.10	0.36	-48.9	0.68
	85°C	1.1 ± 0.1	0.37	-49.3	1.5 ± 0.3	1.28 ± 0.20	0.36	-49.2	0.51
	90°C	0.4 ± 0.1	0.70	-12.7	0.3 ± 0.2	0.87 ± 0.37	0.72	-10.0	0.80

Parameters were estimated only in each a_w and temperature condition, and only compared within each row.

Table 5.7 Parameter estimates (mean ± standard error) for the log-linear and Weibull models, root mean squared errors (RMSE), and AIC_c values for almond kernels, meal, and butter.

Products		Log-linear model			Weibull model				
		D-value (min)	RMSE (log CFU/g)	AIC_c	δ (min)	p	RMSE (log CFU/g)	AIC_c	Relative likelihood of log-linear over Weibull model (per AIC_c)
Almond kernels									
0.25 a_w	80°C	17.6 ± 1.7	0.62	-21.1	18.4 ± 0.3	1.03 ± 0.24	0.63	-18.3	0.80
	85°C	10.1 ± 1.1	0.66	-17.1	12.2 ± 4.2	1.15 ± 0.30	0.67	-14.7	0.77
	90°C	6.1 ± 0.7	0.67	-16.2	3.2 ± 1.7	0.71 ± 0.17	0.64	-16.4	0.47
0.45 a_w	80°C	24.8 ± 3.1	0.89	-1.3	11.9 ± 8.2	0.70 ± 0.19	0.87	-0.4	0.61
	85°C	11.9 ± 1.0	0.51	-25.9	9.2 ± 3.2	0.86 ± 0.16	0.51	-23.6	0.76
	90°C	5.6 ± 0.7	0.71	-12.2	3.3 ± 2.0	0.73 ± 0.21	0.70	-10.9	0.65
0.65 a_w	80°C	11.5 ± 1.5	0.80	-6.4	6.0 ± 3.9	0.71 ± 0.20	0.79	-5.7	0.58
	85°C	3.1 ± 0.7	0.84	-4.1	2.5 ± 1.7	0.82 ± 0.42	0.85	-1.5	0.782
	90°C	1.1 ± 0.1	0.71	-12.2	1.4 ± 0.5	1.20 ± 0.37	0.72	-9.8	0.77

Parameters were estimated only in each a_w and temperature condition, and only compared within each row.

Table 5.7 Parameter estimates (mean ± standard error) for the log-linear and Weibull models, root mean squared errors (RMSE), and AIC_c values for almond kernels, meal, and butter (cont'd).

Products		Log-linear model			Weibull model				
		D-value (min)	RMSE (log CFU/g)	AIC_c	δ (min)	p	RMSE (log CFU/g)	AIC_c	Relative likelihood of log-linear over Weibull model (per AIC_c)
Almond meal									
0.25 a_w	80°C	75.2 ± 4.3	0.23	-74.3	67.6 ± 11.6	0.91 ± 0.12	0.23	-72.1	0.75
	85°C	42.3 ± 2.6	0.35	-48.9	33.5 ± 8.5	0.86 ± 0.13	0.35	-47.4	0.67
	90°C	21.3 ± 1.3	0.46	-37.3	12.6 ± 3.9	0.76 ± 0.11	0.43	-38.9	0.32
0.45 a_w	80°C	48.7 ± 3.7	0.41	-39.7	35.8 ± 10.9	0.82 ± 0.14	0.40	-38.5	0.64
	85°C	23.4 ± 2.0	0.54	-26.1	13.1 ± 5.3	0.73 ± 0.13	0.51	-26.8	0.41
	90°C	9.9 ± 0.6	0.39	-46.0	5.9 ± 1.6	0.75 ± 0.10	0.36	-48.6	0.22
0.65 a_w	80°C	20.1 ± 0.9	0.29	-60.0	16.4 ± 3.1	0.88 ± 0.09	0.28	-58.8	0.66
	85°C	7.4 ± 0.4	0.35	-51.3	5.4 ± 1.2	0.83 ± 0.10	0.34	-51.6	0.46
	90°C	2.7 ± 0.1	0.14	-99.9	2.8 ± 0.3	1.03 ± 0.08	0.15	-97.4	0.78

Parameters were estimated only in each a_w and temperature condition, and only compared within each row.

Table 5.7 Parameter estimates (mean ± standard error) for the log-linear and Weibull models, root mean squared errors (RMSE), and AIC_c values for almond kernels, meal, and butter (cont'd).

Products		Log-linear model			Weibull model				
		D-value (min)	RMSE (log CFU/g)	AIC_c	δ (min)	p	RMSE (log CFU/g)	AIC_c	Relative likelihood of log-linear over Weibull model (per AIC_c)
Almond butter									
0.25 a_w	80°C	61.6 ± 5.2	0.42	-42.1	62.3 ± 16.9	1.01 ± 0.21	0.43	-39.3	0.80
	85°C	36.0 ± 1.7	0.32	-56.3	23.7 ± 5.0	0.79 ± 0.08	0.29	-59.3	0.18
	90°C	18.4 ± 0.7	0.33	-54.3	13.3 ± 2.6	0.85 ± 0.08	0.32	-55.6	0.35
0.45 a_w	80°C	48.9 ± 7.2	0.81	-6.0	19.8 ± 15.3	0.61 ± 0.20	0.79	-5.8	0.53
	85°C	23.6 ± 2.6	0.73	-12.2	11.6 ± 6.8	0.69 ± 0.17	0.71	-12.1	0.52
	90°C	8.0 ± 0.7	0.59	-23.2	6.5 ± 2.6	0.88 ± 0.20	0.60	-20.8	0.77
0.65 a_w	80°C	13.7 ± 0.8	0.42	-41.8	10.1 ± 2.7	0.85 ± 0.11	0.41	-41.0	0.60
	85°C	4.7 ± 0.3	0.45	-36.7	4.2 ± 1.1	0.93 ± 0.13	0.46	-34.1	0.78
	90°C	1.7 ± 0.1	0.42	-39.9	1.1 ± 0.3	0.79 ± 0.10	0.40	-41.5	0.30

Parameters were estimated only in each a_w and temperature condition, and only compared within each row.

Table 5.8 Parameter estimates (mean ± standard error) for the log-linear and Weibull models, root mean squared errors (RMSE), and AIC_c values for date pieces and date paste.

Products		Log-linear model			Weibull model				
		D-value (min)	RMSE (log CFU/g)	AIC_c	δ (min)	p	RMSE (log CFU/g)	AIC_c	Relative likelihood of log-linear over Weibull model (per AIC_c)
Date pieces									
0.25 a_w	80°C	8.2 ± 1.0	0.55	-27.5	11.5 ± 2.7	1.42 ± 0.41	0.54	-26.5	0.62
	85°C	2.9 ± 0.3	0.57	-23.1	4.5 ± 1.0	1.43 ± 0.29	0.54	-24.0	0.39
	90°C	1.1 ± 0.2	0.91	-0.2	1.9 ± 0.6	1.71 ± 0.66	0.89	0.8	0.61
0.45 a_w	80°C	5.4 ± 0.9	0.92	2.0	5.4 ± 3.2	1.00 ± 0.41	0.95	5.2	0.83
	85°C	3.0 ± 0.7	1.11	9.6	3.5 ± 2.2	1.13 ± 0.57	1.14	12.6	0.82
	90°C	1.2 ± 0.4	1.64	29.7	0.8 ± 1.1	0.73 ± 0.57*	1.67	32.3	0.79
0.65 a_w	80°C	5.9 ± 1.4	1.23	13.7	8.7 ± 5.0	1.41 ± 0.78*	1.25	16.3	0.78
	85°C	2.8 ± 0.4	0.93	1.7	1.8 ± 1.2	0.79 ± 0.26	0.93	3.6	0.73
	90°C	1.0 ± 0.1	0.98	4.0	0.8 ± 0.5	0.87 ± 0.31	1.00	6.7	0.80

Parameters were estimated only in each a_w and temperature condition, and only compared within each row.

* This value is not significantly different ($\alpha = 0.05$) from zero.

Table 5.8 Parameter estimates (mean ± standard error) for the log-linear and Weibull models, root mean squared errors (RMSE), and AIC_c values for date pieces and date paste (cont'd).

Products		Log-linear model			Weibull model				
		D-value (min)	RMSE (log CFU/g)	AIC_c	δ (min)	p	RMSE (log CFU/g)	AIC_c	Relative likelihood of log-linear over Weibull model (per AIC_c)
Date pieces									
0.25 a_w	80°C	32.6 ± 4.7	0.42	-39.9	27.4 ± 9.8	0.82 ± 0.27	0.43	-37.5	0.76
	85°C	16.4 ± 2.4	0.44	-39.8	9.6 ± 4.5	0.61 ± 0.19	0.42	-39.9	0.48
	90°C	5.2 ± 0.7	0.45	-34.9	5.2 ± 1.6	1.01 ± 0.36	0.46	-32.1	0.81
0.45 a_w	80°C	14.5 ± 1.4	0.23	-68.6	12.9 ± 2.5	0.83 ± 0.18	0.23	-66.7	0.72
	85°C	7.5 ± 1.4	0.46	-36.4	7.8 ± 2.3	1.06 ± 0.48	0.47	-33.6	0.80
	90°C	3.2 ± 1.5	0.63	-18.2	3.2 ± 2.1	0.71 ± 0.75*	0.64	-15.5	0.79
0.65 a_w	80°C	5.2 ± 0.6	0.54	-27.2	3.8 ± 1.7	0.80 ± 0.21	0.54	-25.3	0.72
	85°C	1.8 ± 0.2	0.78	-7.6	1.0 ± 0.6	0.75 ± 0.19	0.77	-6.6	0.62
	90°C	0.8 ± 0.2	0.90	3.3	0.5 ± 0.5	0.76 ± 0.43*	0.93	7.0	0.86

Parameters were estimated only in each a_w and temperature condition, and only compared within each row.

* This value is not significantly different ($\alpha = 0.05$) from zero.

5.2.4 Secondary model

The GLM analyses, reported above, indicated that temperature, product structure, and a_w had an effect on *Salmonella* inactivation rates. However, the product structure did not have a consistent effect on inactivation of *Salmonella*. For example, product structure did not impact ($P > 0.05$) *Salmonella* thermal resistance (Table 5.6) in wheat products at 0.45 and 0.65 a_w at any of the temperatures (D-values were compared via 95% CI). *Salmonella* thermal resistance in almond meal and almond butter at 0.25 and 0.45 a_w also were equivalent ($P > 0.05$) for all of the temperatures. Because the dates were only fabricated into paste, giving a mix of two and three levels of product structure, the product structure could not be calculated as a model parameter in the secondary model (log-linear/Bigelow-type model; Equation 21). Instead log-linear/Bigelow-type model parameters were estimated for each of the product types and structures.

Reduction of *Salmonella* populations during thermal come-up time exceeded 3 logs in some cases (Appendix J). To reduce the impact of the varying time 0 populations on model parameters, normalized survivor data (log N/N_0) were used to estimate model parameters in all products for the secondary model.

5.2.4.1 Reference conditions

Optimization of the reference conditions (T_{ref} and $a_{w, ref}$) for each model was required before fitting the models (Dolan et al., 2013). Additionally, it is almost impossible to estimate other parameters (i.e., Z_T and Z_{aw}) when the reference conditions were not close to optimum references, due to the high parameter correlation (Schwaab and Pinto, 2007).

The reference conditions (Figure 5.4) for each of the models generally were near the middle of the temperature and a_w ranges for all of the products. The modified-Bigelow model of wheat

flour from Smith et al. (2016) also supported the mid-range for the reference conditions. However, Datta (1993) concluded that the reference temperature should be very close to the maximum experimental temperature. The difference in the present results (Table 5.9) may have been influenced by the static vs. dynamic experimental temperatures.

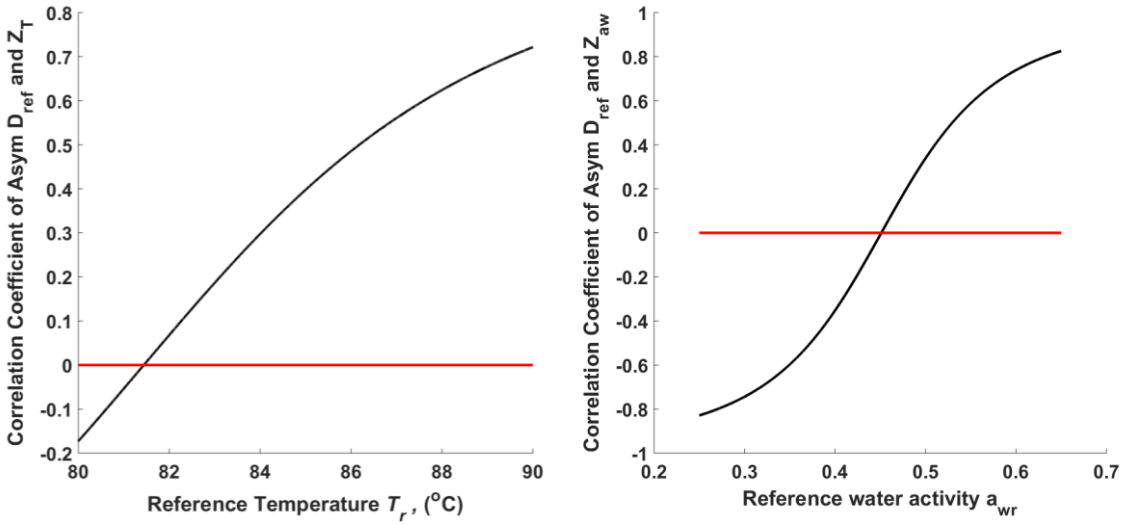

Figure 5.4 Example of reference conditions for the log-linear/Bigelow-type model of almond kernels.

5.2.4.2 Model evaluation

The log-linear/Bigelow-type model parameters for each of the products were estimated using a fixed reference condition. The RMSE and AIC_C values (Table 5.9) indicate that the secondary model for almond meal performed the best across all product types. A model for date pieces provided the highest RMSE and AIC_C, indicating the uncertainty and potential to overfit a

model, respectively. The highest individual parameter relative error for all of the models also was from the date pieces (35% for Z_{aw}).

Table 5.9 Parameter estimates (mean ± standard error) for the log-linear/Bigelow-type models (secondary models), relative error (%), root mean squared error (RMSE), and AIC_c values.

Products	Reference Conditions	Parameter	Estimate	Relative Error (%)	RMSE	AIC_c
Wheat kernels	T_{ref} = 83.9°C	Dref (min)	3.78 ± 0.05	1.4	0.60	-242.79
	$a_{w, ref}$ = 0.493	Z_T (°C)	12.0 ± 0.21	1.8		
		Z_{aw}	0.471 ± 0.008	1.7		
Wheat meal	T_{ref} = 83.1°C	Dref (min)	5.57 ± 0.11	2.0	0.75	-133.17
	$a_{w, ref}$ = 0.466	Z_T (°C)	11.2 ± 0.29	2.6		
		Z_{aw}	0.388 ± 0.008	2.1		
Wheat flour	T_{ref} = 82.7°C	Dref (min)	6.19 ± 0.22	3.6	1.05	27.24
	$a_{w, ref}$ = 0.472	Z_T (°C)	9.88 ± 0.36	3.6		
		Z_{aw}	0.333 ± 0.010	2.9		
Almond kernels	T_{ref} = 81.4°C	Dref (min)	14.6 ± 0.50	3.4	0.97	-6.62
	$a_{w, ref}$ = 0.451	Z_T (°C)	16.2 ± 0.84	5.2		
		Z_{aw}	1.29 ± 0.181	14.0		
Almond meal	T_{ref} = 85.2°C	Dref (min)	17.5 ± 0.22	1.3	0.47	-357.41
	$a_{w, ref}$ = 0.451	Z_T (°C)	13.8 ± 0.29	2.1		
		Z_{aw}	0.509 ± 0.011	2.1		
Almond butter	T_{ref} = 83.8°C	Dref (min)	15.3 ± 0.30	2.0	0.75	-134.56
	$a_{w, ref}$ = 0.483	Z_T (°C)	12.8 ± 0.35	2.7		
		Z_{aw}	0.428 ± 0.010	2.4		
Date pieces	T_{ref} = 76.3°C	Dref (min)	2.21 ± 0.08	3.6	1.08	38.01
	$a_{w, ref}$ = 0.469	Z_T (°C)	11.7 ± 0.58	5.0		
		Z_{aw}	3.62 ± 1.268	35.0		
Date paste	T_{ref} = 73.6°C	Dref (min)	4.08 ± 0.10	2.5	0.51	-289.45
	$a_{w, ref}$ = 0.543	Z_T (°C)	12.6 ± 0.51	4.0		
		Z_{aw}	0.413 ± 0.013	3.1		

Residual analysis of the fitted model (Figure 5.5-5.7), showed different trends for different products. The best fitting model for almond meal was the log-linear/Bigelow-type, because RMSE and AIC_c were the smallest. Wheat kernels and date pieces showed a RMSE > 1 log (N/N_0), and a high AIC_c, corresponding to the distributed data in Figure 5.5A and Figure 5.7A.

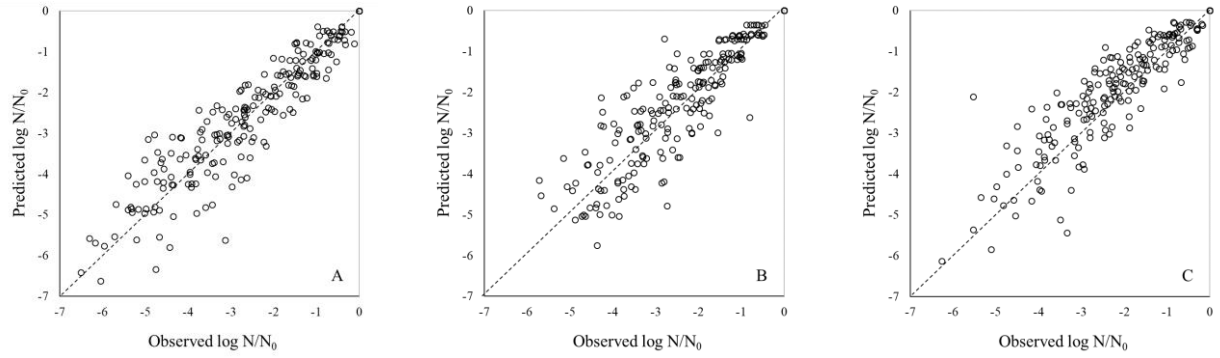

Figure 5.5 Observed and predicted log (N/N_0) for the log-linear/Bigelow-type model for: (A) wheat kernels, (B) wheat meal, and (C) wheat flour.

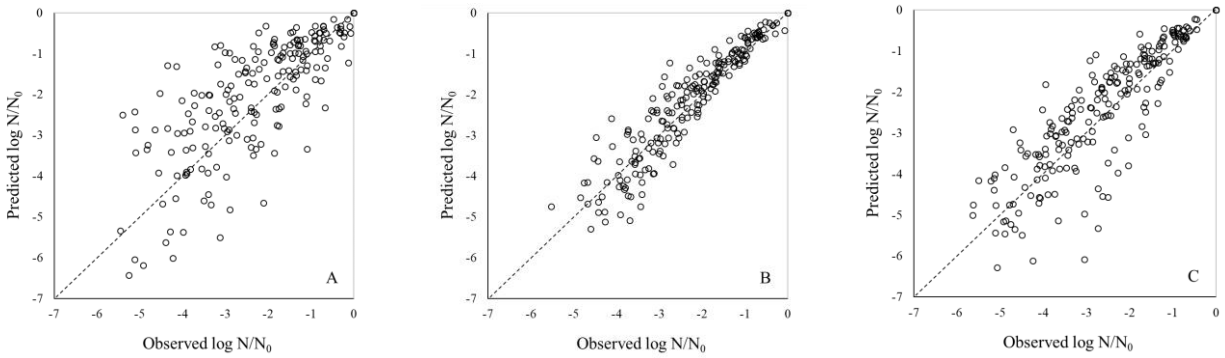

Figure 5.6 Observed and predicted log (N/N_0) for the log-linear/Bigelow-type model for: (A) almond kernels, (B) almond meal, and (C) almond butter.

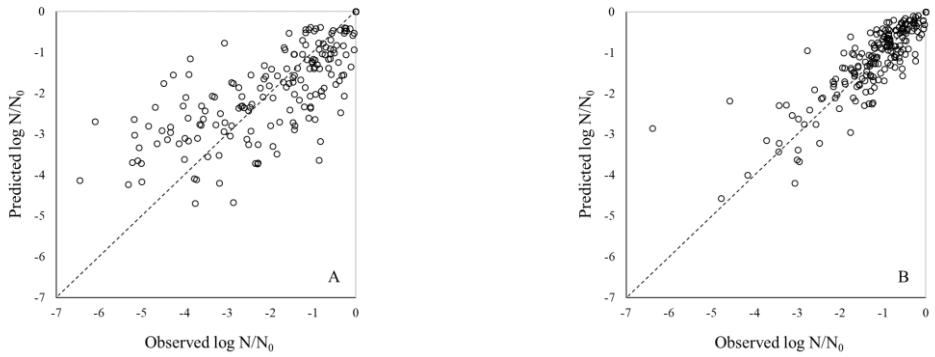

Figure 5.7 Observed and predicted log (N/N_0) for the log-linear/Bigelow-type model for: (A) date pieces and (B) date paste.

When compared within product type, the bias (Table 5.10) was not significantly different ($P > 0.05$; confirmed by ANOVA). All models underestimated the values for all products, except date pieces, which overestimated. The greatest model bias was seen for almond products.

Table 5.10 Model bias for each product from the Bigelow-type models. Negative values indicate underprediction of actual lethality.

Product	Bias (log N/N_0)		
	kernels/pieces	meal	flour/butter/paste
Wheat	-0.02	-0.10	-0.08
Almond	-0.24	-0.11	-0.17
Date	0.03	NA	-0.05

The relationship between the D-value, that were calculated from the log-linear and the log-linear/Bigelow-type models, and a_w (Figure 5.8-5.10) showed some systematic errors, especially in almond products at low temperature and a_w (Figure 5.9). Therefore, the overall model may not be sufficiently accounting for the interactive effect of temperature and a_w on *Salmonella* thermal resistance.

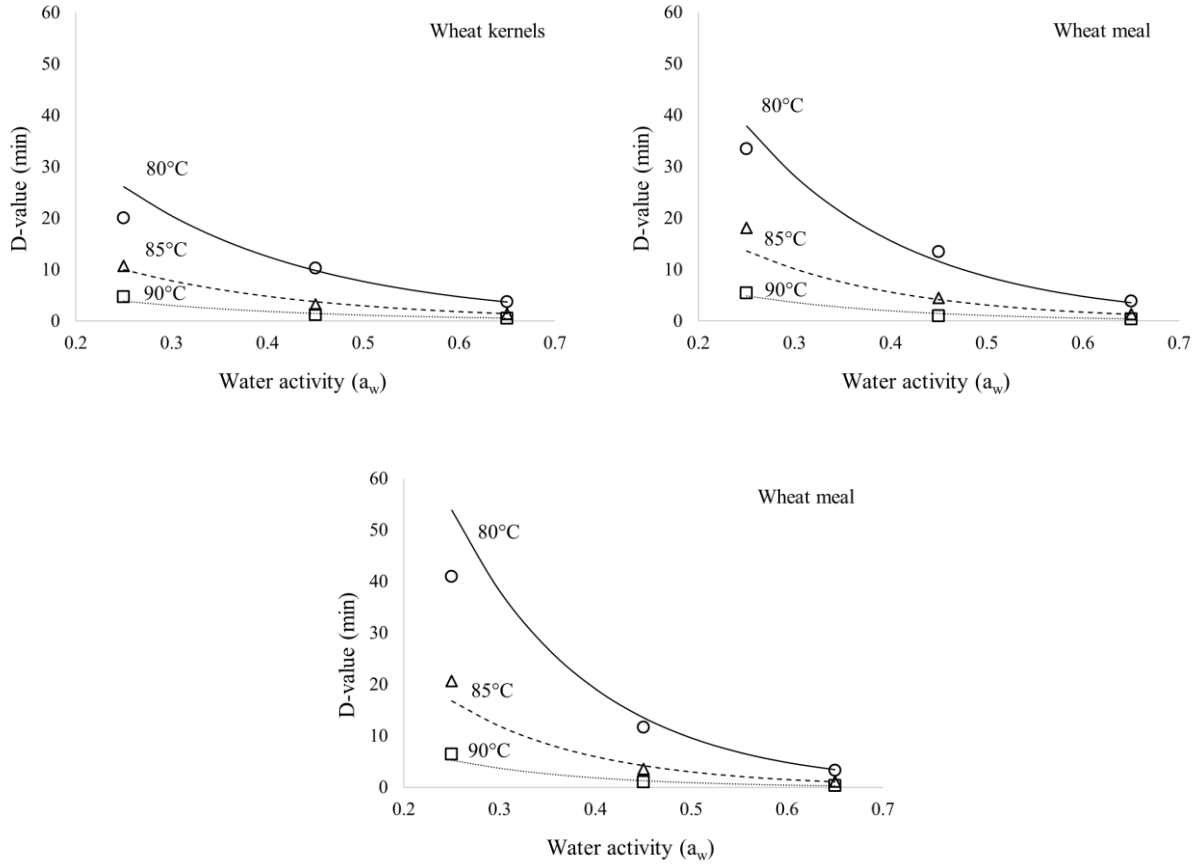

Figure 5.8 Relationship of D-value, estimated from log-linear (symbols) vs log-linear/Bigelow-type (line) models, and a_w for wheat kernels, wheat meal, and wheat flour.

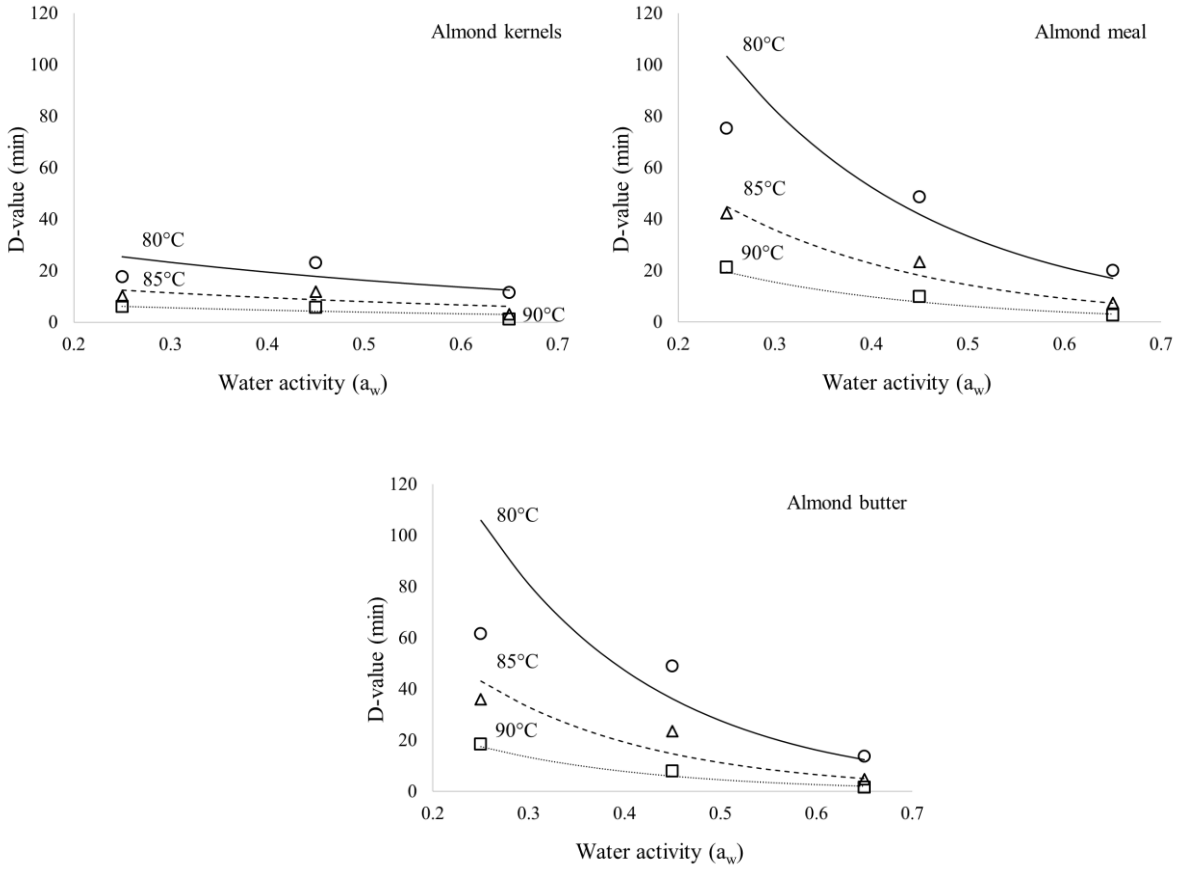

Figure 5.9 Relationship of D-value, estimated from log-linear (symbols) vs log-linear/Bigelow-type (line) models, and a_w for almond kernels, almond meal, and almond butter.

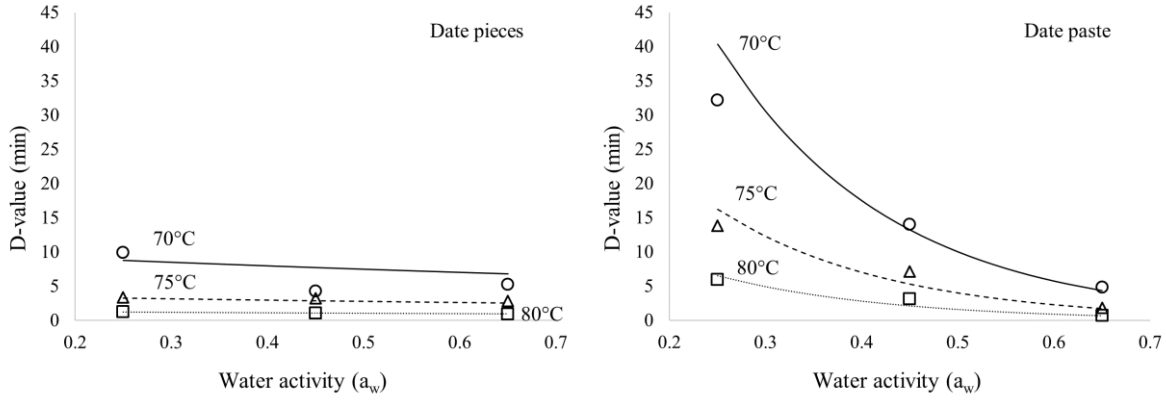

Figure 5.10 Relationship of D-value, estimated from log-linear (symbols) vs log-linear/Bigelow-type (line) models, and a_w for date pieces, and date paste.

Few studies reported Z_T and Z_{aw} based on the Bigelow-type model. Smith et al. (2016) developed secondary model for wheat flour (T_{ref} = 80°C and $a_{w, ref}$ = 0.52), with RMSE of 0.78 log CFU/g. The calculated $D_{80°C, 0.52\ aw}$ for wheat flour in the present study was 8.26 min, which was higher than 2.52 min from Smith et al. (2016). Their Z_T (15.2°C) was also higher than the 9.88°C in this study, but the Z_{aw} (0.33) was similar to value in this study. It should be noted that their experiment temperature was 75-85°C and a_w was 0.310-0.700 a_w. Product composition may also have caused the differences. The higher fat content of whole wheat flour compared to white wheat flour may also responsible for these differences. In addition, the range of temperatures may have had more of an impact than the range of a_w, indicating the differences of Z_T and Z_{aw} in both studies.

Villa-Rojas et al. (2013) also developed a Bigelow-type model for almond kernel flour. Their Z_T (8.28°C) and Z_{aw} (0.187) were considerably lower than in the present study. Their $a_{w, ref}$ was fixed at 1, and they used 121°C for T_{ref}, which was above the experimental temperature range

(56 – 80°C). Moreover, the a_w range (0.65 to 0.95) was almost entirely above that of the present study, which would be expected to affect model parameters.

Other recent studies reported the similar multivariable inactivation models for non-isothermal and non-isomoisture treatments of low-moisture products. Using similar *Salmonella* inactivation model for pistachios, Casulli (2016) reported that their Z_T (37.1°C) was higher than the present 16.2°C for almond kernels, but the Z_{aw} (0.26) was lower than the 1.29 Z_{aw} in this study. While these results suggest that temperature changes may have less of an influence in non-isothermal treatments, a_w changes had more of an influence when the product moisture changed dramatically during thermal processing. Also, the difference in product composition may have had an impact on the estimated model parameters.

Jeong et al. (2009) developed the modified MSU inactivation model based on process and dew point temperature for almonds. For the dry treatment (5% MC in oven), the Z_T value (14.68°C) was close to 16.24°C. Garcés-Vega (2017) further developed the modified MSU model for low- and high-humidity values. The Z_T (69.1°C and 106°C) were much higher than in this study. The humidity of the process conditions may have caused the difference between the model parameters. Unfortunately, another parameter based on water properties cannot be compared due to the difference in the design of the experiments (i.e., closed containers in the present study vs. open-air heating in Jeong et al. 2009)

The SSC analysis (Figure 5.11) shows the correlation between model parameters. The SSC shows a similar result for all of the products (Appendix K); however, differences in the magnitude of SSC over time can be seen. Results also suggest a correlation between Z_T and Z_{aw}. Additional analyses examined the correlation between Z_T and Z_{aw} (Figure 5.12).

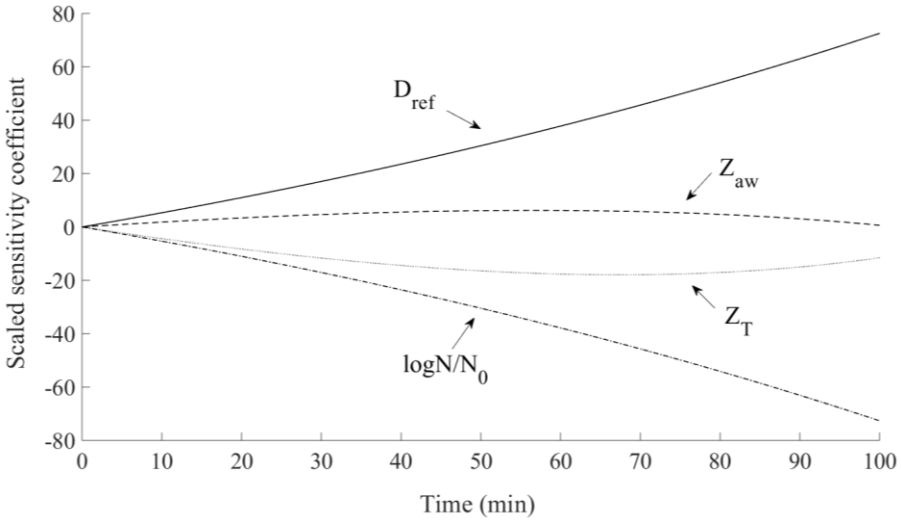

Figure 5.11 Example of SSC for the log-linear/Bigelow-type model of almond kernels.

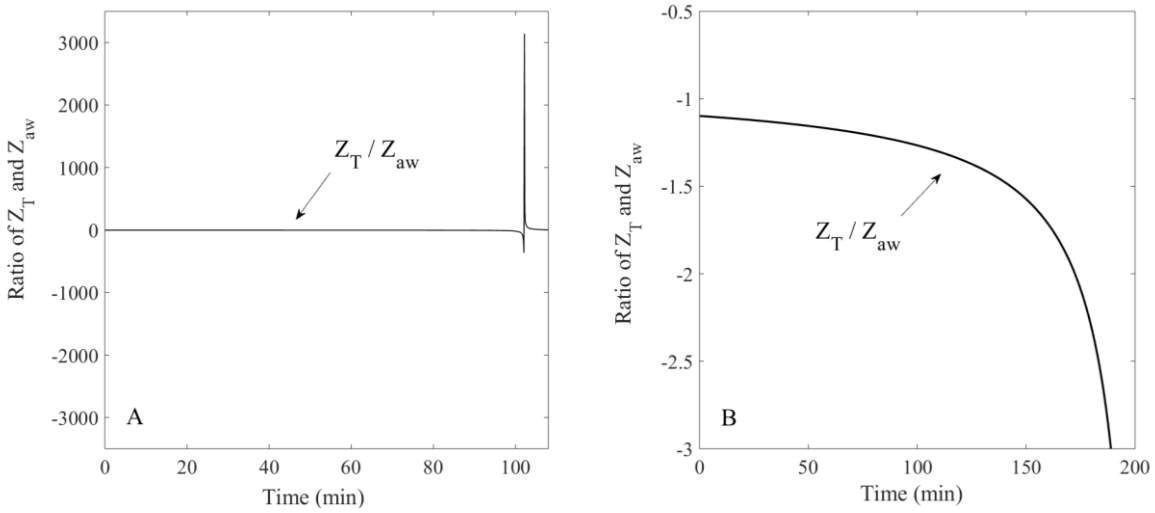

Figure 5.12 Example of SSC for the log-linear/Bigelow-type model for: (A) almond kernels and (B) almond meal.

Almond kernels and date pieces exhibited similar correlation trends between Z_T and Z_{aw} (Figure 5.12A and Appendix K). Wheat products, almond meal, almond butter, and date paste also showed similar trends as in Figure 5.12B (See Appendix K).

Results suggest a significant correlation between Z_T and Z_{aw} in almond kernels and date pieces. However, the relative errors of Z_{aw} for almond kernels and date pieces were relatively high (14% and 35%, respectively). Additionally, the Z_{aw} values were larger than the actual a_w range (0-1). These results suggest that temperature has a greater impact on the D-value in large particle samples, confirming the relationship between Z_T and Z_{aw} (Figure 5.13).

For the smaller-particle samples (wheat products, almond meal, almond butter, and date paste), the Z_T and Z_{aw} relative error results indicated that the two factors were correlated at the beginning of the simulated experiment, but then became uncorrelated when the experiment was completed (Appendix K, Figure K.2-K.6 and K.8). Additionally, the relationship between Z_T and Z_{aw} (Figure 5.13) were clumped together in the figure. This result suggests that particle size (product structure) has an impact on the products corresponding D-value.

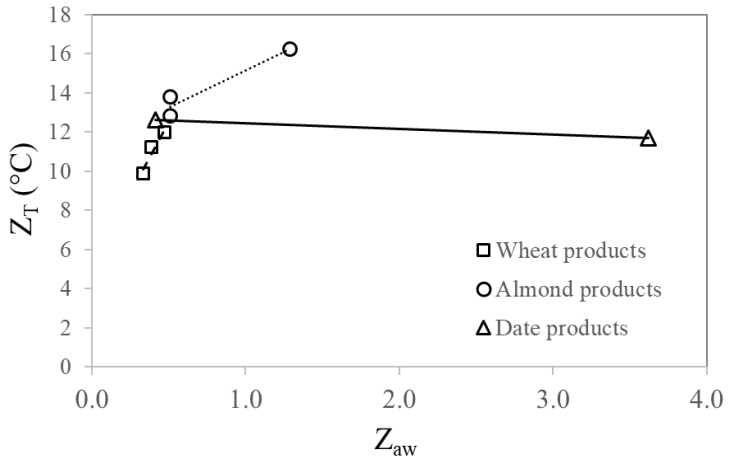

Figure 5.13. Relationship of Z_{aw} and Z_T (°C) for all products.

5.2.5 Water activity effects

The thermal resistance of *Salmonella* is greater at lower a_w than at higher a_w values. During thermal treatments, the a_w of products was changing as the temperature increased. This temperature-induced change in a_w values during heating may affect the thermal resistance of *Salmonella* (Syamaladevi et al., 2016a).

Water activity of the wheat and almond products increased ($P < 0.05$) after heating to 80°C (Table 5.11), but the $a_{w,\ 80°C}$ decreased ($P < 0.05$) for the date products. Syamaladevi et al. (2016a) reported that the a_w of wheat flour at 0.45 $a_{w,\ 25°C}$ increased to 0.80 after heating to 80°C, which was higher than the 0.650 in this study. Tadapaneni et al. (2017) reported a $a_{w,\ 80°C}$ for wheat flour (initial ~0.45 $a_{w,\ 25°C}$) at 0.73 when using test cells. Differences in measurement methodology may have impacted the $a_{w,\ 80°C}$ results. Samples tested by Syamaladevi et al. (2016a) were measured using a vapor sorption analyzer, whereas Tadapaneni et al. (2017) were measured by a RH sensor within the test cell, as in the present study. Differences in wheat composition between these studies may have also account for the change in a_w.

Table 5.11 Water activity values (± standard deviation) at 25 and 80°C for wheat, almond, and date products

	Products	Measured a_w	
		at 25°C	at 80°C
0.25 a_w	Wheat kernels	0.256 ± 0.001^{B}	0.455 ± 0.000^{A}
	Wheat meal	0.256 ± 0.001^{B}	0.475 ± 0.007^{A}
	Wheat flour	0.254 ± 0.001^{B}	0.465 ± 0.021^{A}
0.45 a_w	Wheat kernels	0.456 ± 0.001^{C}	0.670 ± 0.007^{A}
	Wheat meal	0.444 ± 0.001^{D}	0.670 ± 0.000^{A}
	Wheat flour	0.445 ± 0.001^{D}	0.650 ± 0.007^{B}
0.65 a_w	Wheat kernels	0.642 ± 0.004^{B}	0.780 ± 0.007^{A}
	Wheat meal	0.651 ± 0.000^{B}	0.795 ± 0.000^{A}
	Wheat flour	0.651 ± 0.004^{B}	0.795 ± 0.028^{A}
0.25 a_w	Almond kernels	0.249 ± 0.001^{C}	0.380 ± 0.007^{A}
	Almond meal	0.254 ± 0.001^{C}	0.415 ± 0.007^{A}
	Almond butter	0.249 ± 0.001^{C}	0.325 ± 0.007^{B}
0.45 a_w	Almond kernels	0.448 ± 0.002^{D}	0.525 ± 0.000^{B}
	Almond meal	0.442 ± 0.000^{D}	0.550 ± 0.000^{A}
	Almond butter	0.451 ± 0.000^{D}	0.465 ± 0.000^{C}
0.65 a_w	Almond kernels	0.644 ± 0.001^{A}	0.665 ± 0.000^{A}
	Almond meal	0.654 ± 0.000^{A}	0.690 ± 0.007^{B}
	Almond butter	0.662 ± 0.000^{A}	0.650 ± 0.007^{A}
0.25 a_w	Date pieces	0.254 ± 0.005$^{B, C}$	0.280 ± 0.014^{B}
	Date paste	0.238 ± 0.000^{C}	0.320 ± 0.000^{A}
0.45 a_w	Date pieces	0.456 ± 0.001^{A}	0.435 ± 0.007^{B}
	Date paste	0.459 ± 0.001^{A}	0.440 ± 0.000^{B}
0.65 a_w	Date pieces	0.648 ± 0.001^{A}	0.600 ± 0.014^{B}
	Date paste	0.642 ± 0.001^{A}	0.610 ± 0.000^{B}

Within the same water activity and product type, means sharing a common superscript letter were not significantly different ($\alpha = 0.05$).

Syamaladevi et al. (2016a) also reported an $a_{w,\ 80°C}$ for peanut butter (initial ~0.45 $a_{w,\ 25°C}$) of 0.04, which was much lower than the almond butter ($a_{w,\ 80°C}$ = 0.465) in this study. In addition, Anderson et al. (2017) reported the $a_{w,\ 80°C}$ of a protein-fat blend (43% protein and 56% fat, and $a_{w,\ 40°C}$ of 0.341) to be 0.366. Again, these differences may be caused by variation in methodology and product compositions. Moreover, the heating time to 80°C in this study (37 min) was considerably faster than for Syamaladevi et al. (2016a) (samples were reported to equilibrate for 2 weeks before measurement). Oil separation in these almond and peanut butter during longer equilibration processes could also have impacted the measurement.

In this study, $a_{w,\ 80°C}$ was similar ($P < 0.05$) amongst all wheat products at 0.25 $a_{w,\ 25°C}$. However, *Salmonella* thermal resistance in wheat meal and flour at 0.25 $a_{w,\ 25°C}$ was higher ($P < 0.05$) than wheat kernels. Corresponding with the almond product results, the $a_{w,\ 80°C}$ for almond butter was equivalent to $a_{w,\ 25°C}$ ($P > 0.05$) when the initial $a_{w,\ 25°C}$ was 0.65; however, *Salmonella* thermal resistance was greater in almond meal ($P < 0.05$) than in almond butter and almond kernels. Additionally, *Salmonella* thermal resistance in date paste (0.25 $a_{w,\ 25°C}$) was greater ($P < 0.05$) than on date pieces, but the $a_{w,\ 80°C}$ was greater ($P < 0.05$).

In contrast, the $a_{w,\ 80°C}$ of wheat product (0.65 $a_{w,\ 25°C}$) was not significantly different ($P > 0.05$) amongst the product structures, and *Salmonella* thermal resistance was equivalent ($P > 0.05$) for all wheat products. Additionally, *Salmonella* thermal resistance among date products (0.45 and 0.65 $a_{w,\ 25°C}$) was equivalent ($P > 0.05$), and the $a_{w,\ 80°C}$ of date piece and date paste was not different ($P > 0.05$). These results indicate that *Salmonella* thermal resistance may be partially affected by a_w at each of the processing temperatures; however, some of the inconsistencies would imply that high-temperature a_w cannot be the sole explanation for observed differences in *Salmonella* thermal resistance across product types and different structures.

Additionally, the adsorption isotherm may have an impact on *Salmonella* thermal resistance. Garcés-Vega (2017) reported that the sensitivity of *Salmonella* on almonds was more likely influenced by moisture content than by a_w. However, his study was non-isothermal and non-isomoisture, which was different from this study. The measured a_w of the source wheat, almonds, and dates, as originally acquired, in the present study were 0.351, 0.504, and 0.709 a_w, respectively. Therefore, the 0.25 a_w wheat products, 0.25 and 0.45 a_w almond products, and all date products were in a desorption state, and all other products were in an adsorption state when tested.

5.2.6 Product type and structure effects

Salmonella thermal resistance is influenced by the composition of the food matrix. In this study, the impact of product composition (Table 5.12) including the sugar profile of dates (Table 5.13) on *Salmonella* thermal resistance was evaluated.

Wheat and dates were higher in carbohydrates than almonds, with dates contain 63.3% sugar. Almonds were highest in fat among all three products. These compositional differences (carbohydrate, fat, and sugar) may influence thermal resistance of *Salmonella*. Additionally, the date moisture content was 232% higher than wheat, and 475% higher than almonds, which could be the most significant factor leading to lower thermal resistance.

Table 5.12 Composition results for almond, wheats, and date.

Component	% Content			Reference method
	Wheat	Almond	Date	
Ash	1.7	3.2	1.9	AOAC 920.153
Carbohydrate	81.4	24.0	71.4	Calculation*
Protein	7.8	25.7	2.8	AOAC 922.06
Fat	2.0	43.0	0.3	AOAC 950.46
Moisture	7.1	4.1	23.6	AOAC 992.15

* The % content of carbohydrate was the percentage of solids that were not protein or fat.

Table 5.13 Sugar profile for dates using high-performance liquid chromatography (HPLC).

Sugar type	% Content
Fructose	14.7
Glucose	17.0
Lactose	< 0.04
Maltose	< 0.04
Sucrose	< 0.04
Total sugar	31.6

% total of sugar profile of dates was based on 100% total in table 5.12.

DSC results (Table 5.14) showing the thermophysical transitions (Appendix L) during thermal processing suggested that a structure transition in almond butter, which may impact *Salmonella* thermal resistance. In addition, the characteristic peaks observed in date paste resulted from the melting of sugar, which likely could decrease *Salmonella* thermal resistance.

Table 5.14 DSC parameters (± standard deviation) for 0.25, 0.45, and 0.65 a_w wheat flour, almond butter, and date paste.

Products		Glass transition temperature (°C)			Characteristic temperature and enthalpy		
		T_{gi}	T_g	T_{ge}	T_{po} (°C)	T_p (°C)	ΔH (J/g)
Wheat flour	0.25 a_w	NA[1]	NA	NA	NA	NA	NA
	0.45 a_w	NA	NA	NA	NA	NA	NA
	0.65 a_w	NA	NA	NA	NA	NA	NA
Almond butter	0.25 a_w	39.3 ± 1.7$^{B, 2}$	41.6 ± 0.6^{B}	44.0 ± 1.2^{B}	NA	NA	NA
	0.45 a_w	46.7 ± 0.5^{A}	48.6 ± 1.0^{A}	49.6 ± 1.0^{A}	NA	NA	NA
	0.65 a_w	44.8 ± 0.2^{A}	45.5 ± 0.9^{A}	45.8 ± 0.4$^{A, B}$	NA	NA	NA
Date paste	0.25 a_w	NA	NA	NA	66.4 ± 3.4^{A}	80.9 ± 1.7^{A}	11.9 ± 0.7^{B}
	0.45 a_w	NA	NA	NA	72.2 ± 0.3^{A}	83.0 ± 0.0^{A}	18.0 ± 0.6^{A}
	0.65 a_w	NA	NA	NA	51.7 ± 0.2^{B}	70.9 ± 0.2^{B}	9.2 ± 0.7^{B}

[1] NA means values could not be estimated due to no thermal transitions.

[2] Within a column, values with a common superscript letter were not significantly different (α = 0.05).

5.2.6.1 Wheat products

Salmonella was most thermally resistant among the wheat products at the $D_{80°C,\ 0.25\ aw}$ in wheat flour. Grinding the wheat kernels into meal and flour increased ($P < 0.05$) *Salmonella* thermal resistance at 0.25 a_w. However, *Salmonella* thermal resistance in the meal and flour were not significantly different from each other regardless of temperature or a_w. Moreover, *Salmonella* exhibited similar thermal resistance $P > 0.05$) at 0.45 and 0.65 a_w, regardless of the product structure.

The DSC results (Table 5.14) show no thermal transitions for wheat flour regardless of a_w or temperature; therefore, it appears unlikely that any such thermophysical changes are affecting *Salmonella* thermal resistance.

However, at 0.25 a_w, *Salmonella* thermal resistance was higher ($P < 0.05$) in wheat flour compared to wheat kernels for all temperatures. This result indicates that product structure has a greater influence on *Salmonella* thermal resistance in wheat products at low a_w, even though the mechanism for this effect is unknown.

Unfortunately, previous studies did not report *Salmonella* thermal resistance on wheat kernels or in wheat meal. Syamaladevi et al. (2016a) reported $D_{80°C,\ 0.45\ aw}$ of 6.9 min for wheat flour, which was lower than the 11.6 min in this study. Smith et al. (2016) reported a $D_{80°C,\ 0.427\ aw}$ of 5.5 min for wheat flour which also was lower than in this study. The Smith et al. (2016) D-value for wheat flour would be expected to be higher than that of Syamaladevi et al. (2016a) and this study due to the lower a_w, but results indicated the opposite. Syamaladevi et al. (2016a) inoculated wheat flour with a *Salmonella* cocktial, but Smith et al. (2016) and this study used a single strain of *Salmonella* Enteritidis PT 30 as in this study. Another difference was the overall composition of the white wheat flour used. Syamaldevi et al. (2016a) and Smith et al. (2016) used

different commercial brands of wheat flour brands, where in this study whole wheat flour was produced by grinding the wheat kernels in a mill and not removing the wheat bran. The results reinforce the notion that *Salmonella* thermal resistance is impacted by product composition.

5.2.6.2 Almond products

Within almond products, *Salmonella* was most thermally resistant in almond meal regardless of temperature or a_w. The fabrication process for turning almond kernels into meal and butter increased *Salmonella* thermal resistance by 325% at 0.25 a_w and T = 80°C (Table 5.7). In comparison, *Salmonella* thermal resistance in almond meal and butter was equivalent ($P > 0.05$) for all temperatures at 0.25 and 0.45 a_w. These results suggest that *Salmonella* thermal resistance on/in almond products was influenced by more product structure at high a_w.

At 0.65 a_w, *Salmonella* thermal resistance in almond meal was greater ($P < 0.05$) than in/on the butter and kernels for all temperatures. Almond butter at 0.65 a_w is higher in moisture and therefore lower in fat content. Hence, the reduction in fat content at the higher a_w may have contributed to the decreased in thermal resistance (He et al., 2011).

The DSC results (Table 5.14) show that the phase transition for almond butter occurred between 42 - 48°C, regardless of a_w. Glass transition temperatures (T_g) for almond butter at 0.45 and 0.65 a_w were higher ($P < 0.05$) than at 0.25 a_w, but *Salmonella* thermal resistance of almond meal and butter at 0.45 a_w were not significantly different ($P > 0.05$), possibly due to denaturation of almond protein at 80°C (Amirshaghaghi et al., 2017). Based on these results, and experimental observation during testing, the almond butter changed from a viscous-liquid to a semi-solid product, which behaved similarly to almond meal.

Almond kernels have been subjected to various thermal pasteurization processes, using moist-air oven (Jeong et al., 2009), hot water (Harris et al., 2012), or hot oil (Abd et al., 2012; Du et al., 2010). The $D_{80°C}$ for one hot water treatment was 0.75 min (Harris et al., 2012) and the hot oil treatment reducing *Salmonella* by 4 logs in 1.2 min at 121°C (Du et al., 2010). In the present study, the $D_{80°C}$ was 23.1 min.

Only one study reported the D-value for almond meal. Villa-Rojas et al. (2013) reported a $D_{80°C, 0.601\ aw}$ of 1.63 min, which was much lower than the 20.1 min D-value (0.65 a_w) in the present study. The almond meal of Villa-Rojas et al. (2013) was inoculated after fabrication, whereas the opposite occurred in this study. Therefore, the variable D-values can be partially explained by differences in the inoculation methods (See Chapter 3).

In comparing the almond butter results with prior peanut butter work, Li et al. (2014a) and He et al. (2013) used peanut butter containing 48 and 49% fat, which is close to the almond butter in this study. Li et al. (2014a) reported $\delta_{80°C}$, $\delta_{85°C}$, $\delta_{90°C}$ values of 1.6, 2.3, and 2.6 min, respectively, whereas He et al. (2013) reported $D_{90°C}$ values at 0.2, 0.4, and 0.6 a_w of 4.8, 3.4, and 2.1 min, respectively. These results indicate that *Salmonella* thermal resistance was greater in almond butter than peanut butter at the temperatures and a_w values tested. As described in Chapter 3, the post-fabrication method of inoculation was likely responsible for the lower thermal inactivation rate.

5.2.6.3 Date products

The fabrication process increased *Salmonella* thermal resistance in date products. The resistance in date paste was higher ($P < 0.05$) than in date pieces at 0.25 and 0.45 a_w; however, product structure did not impact *Salmonella* thermal resistance at 0.65 a_w. This behavior is in

contrast with the fabricated almond product results. Increasing the a_w of date products reduced the effect that structure had on *Salmonella* thermal resistance.

The DSC results also suggest that some thermophysical transitions may affect *Salmonella* thermal resistance in date products. At 0.65 a_w, the sugars in date paste began melting faster than at 0.25 and 0.45 a_w; however, the enthalpy of transition was higher ($P < 0.05$) at 0.45 a_w than at 0.25 and 0.65 a_w. Sucrose, glucose, and fructose generally do not melt at 80°C (Lee et al., 2011); however, the date paste in this study contained more water than their dry system; therefore, date paste was meltable during thermal treatment.

Unfortunately, no prior studies assessed *Salmonella* thermal resistance on dates. In this study, date products contained 63.3% sugar. Mattick et al. (2001) assessed *Salmonella* thermal resistance in a high sugar content (0.65 a_w) broth at 70 - 80°C, reporting the estimated time for a first log reduction of *Salmonella* Typhimurium between 0.9 – 3.6 min, which were similar to those seen for date paste in this study, but lower than for the date pieces.

5.2.6.4 Comparison between similar product structures.

Product structure was evaluated based on particle size. Large-particle (wheat kernels, almond kernels, and date pieces), small-particle (almond meal, wheat meal, and wheat flour), and paste (almond butter and date paste) products were compared using $D_{80°C}$ at different water activities.

In the large-particle comparison (Figure 5.14), a_w did not have a large impact on the D-value for almond kernels or date pieces; however, the D-value decreased ($P < 0.05$) as a_w increased for wheat kernels. For the small-particle comparison, all of the products showed a similar trend; as a_w increased, the thermal resistance of *Salmonella* decreased ($P < 0.05$).

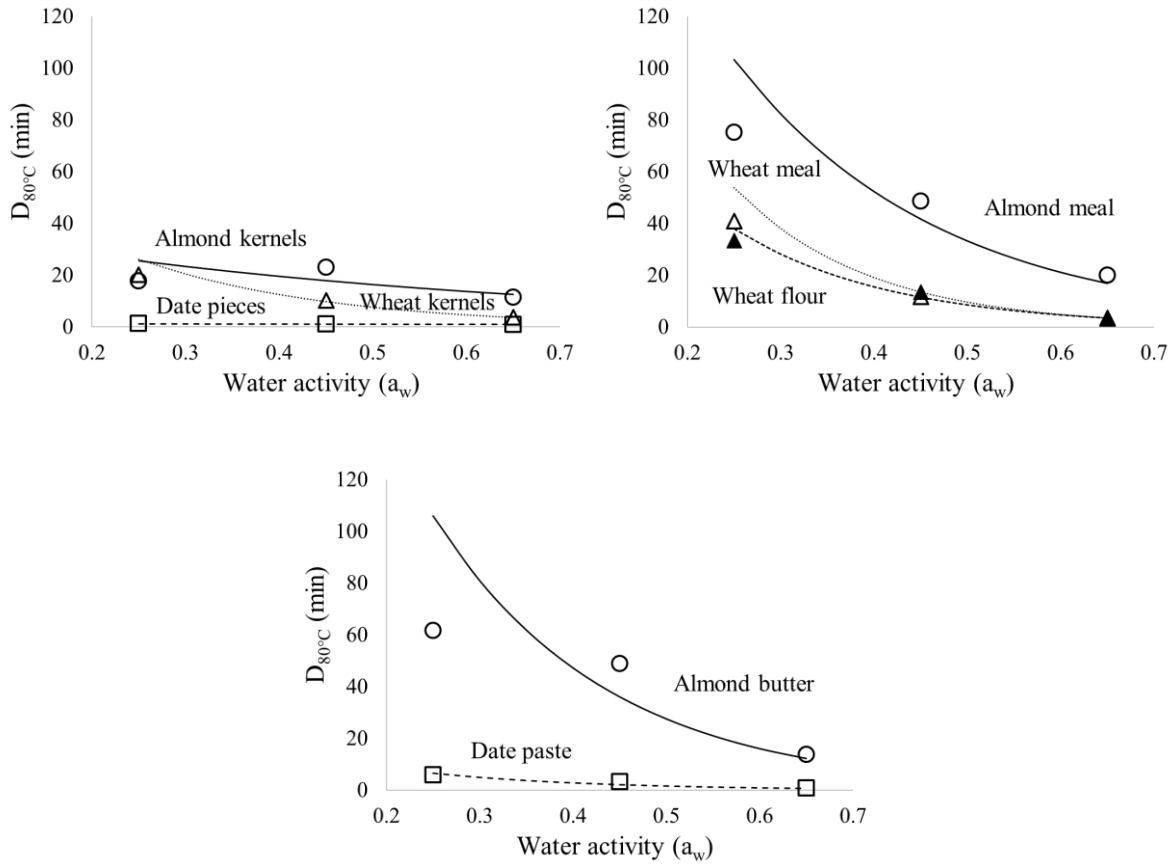

Figure 5.14 Relationship of $D_{80°C}$, estimated from log-linear (dot) vs log-linear/Bigelow-type (line) models, with a_w in similar product structure.

The $D_{80°C,\ 0.45\ aw}$ for all of the products (Table 5.15) were calculated for all products using the model parameters in Table 5.9. When comparing the D-values via 95% CI, product structure had an impact on *Salmonella* thermal resistance across all product types. Standard errors of the estimated D-value from the primary log-linear model were 1.0 – 1.5 min for wheat products, 3.1 – 7.2 min for almond products, and 0.4 – 1.5 min for date products, whereas the standard errors from the log-linear/Bigelow-type model via global regression ranged from 0.06 to 1.19 min. The

range of uncertainty in parameter estimates may be due to product structure effects, causing similar thermal resistance in fabricated products.

Table 5.15 Calculated D-values (± standard error) at 0.45 a_w and 80°C using log-linear/Bigelow-type model.

Products	$D_{80°C, 0.45aw}$ (min)
Wheat kernels	9.80 ± 0.21[A]
Wheat meal	11.30 ± 0.34[B]
Wheat flour	13.35 ± 0.68[C]
Almond kernels	17.83 ± 0.72[A]
Almond meal	41.79 ± 0.97[B]
Almond butter	36.15 ± 1.19[C]
Date pieces	1.08 ± 0.06[A]
Date paste	2.14 ± 0.11[B]

Within a product type, means with common superscript letter were not significantly different (α = 0.05). Statistical analyses were confirmed via 95% CI.

A relationship between Z_T or Z_{aw} and product structure was also seen (Figure 5.15). When particle size decreased, Z_T increased for almond and wheat products. For Z_{aw}, the Z_{aw} tended to decrease to a similar value across all products as the particle size decreased. These results indicate that a_w has a greater influence on the D-value as the particle size decreased.

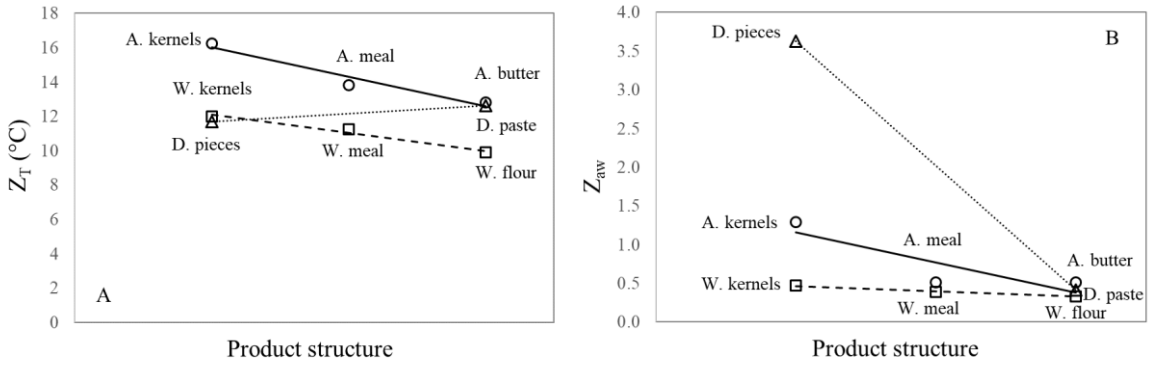

Figure 5.15 Relationship between (A) Z_T and product structure, and (B) Z_{aw} and product structure.

5.3 Conclusion

Process temperature, product structure, and a_w all impacted thermal resistance of *Salmonella* in all three low-moisture product groups (wheat, almond, and dates). Product fabrication increased *Salmonella* thermal resistance for all products. As a_w increased, *Salmonella* thermal resistance decreased at different rates due to variation in product composition (% fat, protein, and carbohydrate). The products that yielded the highest thermal resistance response in *Salmonella* were almond meal and almond butter, likely because the fat content protected *Salmonella* at the high processing temperature. Date pieces yielded the lowest thermal resistance for *Salmonella*, likely due to moisture content of dates being much higher than wheat and almond.

The log-linear model was more-likely-correct model to use in this study for predicting *Salmonella* lethality. The model (log-linear/Bigelow-type) that was developed provided a better understanding of the relationship between temperature and a_w; unfortunately, product structure currently cannot be included as a model term in the secondary model, as it is a discrete state rather than a continuous variable. Regarding the impact of particle size on lethality of *Salmonella*, large particles resulted in a high relative error in the estimated Z_{aw}. For small particles and paste, both

the temperature and a_w influenced the *Salmonella* D-value. The relationship between Z_T and Z_{aw} to these particle sizes showed a similar impact on *Salmonella* inactivation. The general conclusion might be that structure effects are large for the step change in structure due to any grinding, but that finer reductions in particle size (e.g., almond meal to butter, or wheat meal to flour) have much less impact on the thermal resistance of *Salmonella* present in those structure.

6 OVERALL CONCLUSIONS AND RECCOMMENDATIONS

6.1 Other methodological/preliminary work

In addition to the results presented in Chapters 3-5, several other preliminary studies were conducted, with some of their results presented at various conferences. These studies investigated the impact of sample equilibration, kernel surface integrity, and sample containers on *Salmonella* thermal resistance, and are summarized in Appendix M to P.

6.2 Overall Conclusions

Salmonella thermal resistance in fabricated products (meal, butter, flour, and paste) was higher ($P < 0.05$) than in whole products (kernels and pieces) using the pre- and postfabrication protocols, except for wheat products. Using similar inoculation methods (pre-fabrication), *Salmonella* thermal resistance was lower on wheat meal and flour in the inoculation protocols study (Chapter 3) ($P < 0.05$) than in the Chapter 5 study, likely because wheat bran was partially removed in Chapter 3, resulting in a lower fat content.

Fabrication processes also change the microenvironment (e.g., location, attachment) of low-moisture products, which could be the root cause for the observed differences in *Salmonella* thermal resistance, likely because *Salmonella* was located in or attached to specific microenvironments. Contrastingly, *Salmonella* thermal resistance in fabricated wheat products was not significantly different after fabrication, which may be due to a less discrete microenvironment and lower fat and sugar content compared to other products.

Product composition is clearly a very important factor influencing *Salmonella* thermal resistance. Products highest in fat (almonds) yielded the highest thermal resistance among all

fabricated products. In contrast, the high sugar content and moisture content of date products resulted in much lower thermal resistance compared to wheat and almond products.

During long-term storage of almonds, *Salmonella* populations decreased; however, *Salmonella* thermal resistance generally remained unchanged ($P > 0.05$) during long term storage. This information is critically important, to know that the storage age of almonds does not affect thermal resistance and therefore would not affect the approach to thermal process validations in industry.

The relationship between temperature, a_w, and *Salmonella* thermal resistance in low-moisture foods is extremely important. Increasing a_w and temperature decreased *Salmonella* thermal resistance. Additionally, a_w did change after heating and was correlated with *Salmonella* thermal resistance in some of cases; therefore, a_w at the processing temperatures is likely a partial, but incomplete explanation for the observed differences in *Salmonella* thermal resistance.

The resulting primary and secondary models indicated that the log-linear model was the most-likely-correct for low-moisture foods. A Bigelow-type secondary model was developed based on the log-linear model. Based on model parameters, *Salmonella* thermal resistance on the large-particle products (kernels and pieces) were influenced by temperature more than a_w, but resistance on the small-particle products (meal, butter, flour, and paste) was affected by both temperature and a_w. The models also suggest that *Salmonella* on/in all the small-particle and paste products has a similar response with temperature and a_w.

Physical structure also influences *Salmonella* thermal resistance, which is one of the most novel conclusions of this dissertation. During thermal processing, almond butter changed from a

viscous-liquid to a semi-solid, resulting in equivalent D-values for almond meal and butter. Melting of date paste during thermal treatment may have partially increased lethality.

Unfortunately, the relationships between a_w and product structure cannot be directly modeled because product structure is a discrete class variable. In comparing D-values at different a_w, the moisture content of almond products had a greater influence on *Salmonella* resistance in very small particles (butter) than on large particles (kernels). Date paste results also support this conclusion. *Salmonella* in date paste at high a_w showed very low thermal resistance compared to the other products at equivalent a_w levels. Contrastingly, the structure of medium-sized particles (meal and flour) had an impact at low a_w. Also, under very dry conditions, *Salmonella* thermal resistance in wheat meal and flour was greater than on wheat kernels.

Overall, the log-linear/Bigelow-type inactivation models fit well for all products (RMSE from 0.51 to 1.08 log), supporting the robustness of this model form for low-moisture products. All factors (i.e., product type, product structure, temperature, and a_w), except long-term storage, impacted *Salmonella* thermal resistance. This study also demonstrates that inoculation methods and specific product structures should be considered as critical factors when designing process validation studies.

6.3 Future Work

Product structure has been introduced here as a new factor that impacted *Salmonella* thermal resistance in low-moisture foods. However, structure actually encompasses many factors, such as particle size and form (i.e., solid, liquid). Therefore, it is recommended that future work be designed to test particle size as a continuous variable of multiple additional levels, in order to further evaluate whether particle size can be incorporated as a model term.

The physical micro-structure of some low-moisture foods can also change during thermal treatment in some of cases. The a_w increases/decreases when temperature increases. The change in a_w may depend on thermal treatment methods, or also on thermo-physical transitions during heating. The relationship between *Salmonella* thermal resistance and thermophysical properties and a_w at process temperatures is suggested as a topic of further investigation to better understand the impact of product structure during pasteurization.

The sorption-isotherm also impacts *Salmonella* thermal resistance. For example, the moisture content of dates is much higher than that for wheat and almond products at the same a_w. This study was based on the iso-a_w and a isomoisture treatment; however, real processes generally are non-isomoisture. Therefore, it is important to determine the impact of dynamic sorption-isotherms on *Salmonella* thermal resistance, especially in small-particles, due to their higher impact on the resistance.

Lastly, the models presented in this dissertation were based on isothermal/isomoisture data. Therefore, validation of these models in non-iso conditions is essential before they could be applied for commercial process validation. Future work should encompass pilot-scale thermal treatment (e.g., roasting or toasting) of similar products, to quantitatively validate model performance under dynamic conditions.

Overall, *Salmonella* thermal resistance was impacted by changes in product structure, such as particle size, physical state changes, microenvironment properties, and water relationships (i.e., a_w, moisture content, sorption isotherm). Therefore, future research can hopefully advance the science by developing additional novel models that incorporate those fundamental product characteristics, which could lead to an improved general conclusion/theory for product structure effects on *Salmonella* thermal inactivation.

APPENDICES

Appendix A. Survivor Data for the Inoculation Protocol Experiment (Chapter 3)

This appendix shows *Salmonella* inactivation data during thermal treatment at 80°C after samples were inoculated with pre- and post-fabrication protocols and equilibrated to 0.40 – 0.45 a_w.

Table A.1 *Salmonella* inactivation data during isothermal treatment (80°C) for almond meal (~0.4 a_w) using pre-fabrication and post-fabrication inoculation protocols.

	Pre-fabrication			Post-fabrication		
Rep	Time (min)	Log CFU/g	Log N/N_0	Time (min)	Log CFU/g	Log N/N_0
REP 1	0	7.91	0.00	0	8.94	0.00
	16.5	7.51	-0.40	16.5	9.02	0.08
	31.5	6.93	-0.98	31.5	8.45	-0.50
	46.5	6.68	-1.23	46.5	7.73	-1.22
	61.5	6.76	-1.15	61.5	7.71	-1.23
	76.5	6.23	-1.68	76.5	7.06	-1.89
	91.5	6.25	-1.66	91.5	6.83	-2.12
	106.5	6.23	-1.67	106.5	6.82	-2.12
	121.5	6.04	-1.87	121.5	6.47	-2.48
	136.5	5.85	-2.06	136.5	5.95	-3.00
	151.5	5.20	-2.70	151.5	5.94	-3.01
REP 2	0	7.80	0.00	0	8.81	0.00
	16.5	7.24	-0.56	16.5	8.38	-0.43
	31.5	6.83	-0.97	31.5	7.39	-1.42
	46.5	6.51	-1.29	46.5	7.45	-1.35
	61.5	6.02	-1.78	61.5	6.98	-1.83
	76.5	5.84	-1.96	76.5	5.51	-3.30
	91.5	5.46	-2.34	91.5	6.31	-2.50
	106.5	5.00	-2.80	121.5	3.65	-5.16
	121.5	5.34	-2.46	136.5	2.90	-5.91
	136.5	4.66	-3.14	151.5	3.15	-5.66
	151.5	4.98	-2.81			

Table A.1 (cont'd).

	Pre-fabrication			Post-fabrication		
Rep	Time (min)	Log CFU/g	Log N/N_0	Time (min)	Log CFU/g	Log N/N_0
REP 3	0	7.75	0.00	0	9.43	0.00
	16.5	7.38	-0.37	16.5	9.14	-0.29
	31.5	6.60	-1.16	31.5	8.29	-1.14
	46.5	6.34	-1.41	46.5	7.74	-1.68
	61.5	6.02	-1.73	61.5	6.89	-2.54
	76.5	5.52	-2.23	76.5	6.99	-2.44
	91.5	5.45	-2.30	91.5	6.59	-2.84
	106.5	5.34	-2.41	106.5	6.01	-3.42
	113.5	5.53	-2.23	121.5	5.79	-3.64
	136.5	5.45	-2.30	136.5	5.19	-4.24
	151.5	5.49	-2.26	151.5	4.91	-4.52

Table A.2 *Salmonella* inactivation data during isothermal treatment (80°C) for almond butter (~0.4 a_w) using pre-fabrication and post-fabrication inoculation protocols.

	Pre-fabrication			Post-fabrication		
Rep	Time (min)	Log CFU/g	Log N/N_0	Time (min)	Log CFU/g	Log N/N_0
REP 1	0	7.69	0.00	0	9.64	0.00
	16.1	6.88	-0.81	15	8.23	-1.41
	31.1	6.37	-1.32	30	6.55	-3.09
	46.1	5.89	-1.81	45	5.16	-4.48
	61.1	5.51	-2.18	60	4.93	-4.71
	76.1	5.22	-2.48	120	3.22	-6.43
	91.1	4.92	-2.78	135	3.28	-6.36
	106.1	4.92	-2.78	150	3.38	-6.26
	121.1	4.67	-3.02			
	136.1	4.48	-3.22			
	151.1	4.16	-3.53			

Table A.2 (cont'd).

	Pre-fabrication			Post-fabrication		
Rep	Time (min)	Log CFU/g	Log N/N_0	Time (min)	Log CFU/g	Log N/N_0
REP 2	0	7.26	0.00	0	9.39	0.00
	16.1	6.62	-0.63	15	8.17	-1.22
	31.1	5.33	-1.92	30	5.88	-3.51
	46.1	5.36	-1.89	45	5.23	-4.16
	61.1	5.44	-1.82	60	4.54	-4.85
	76.1	5.94	-1.31	75	4.69	-4.70
	86.1	4.94	-2.32	90	4.06	-5.33
	106.1	4.76	-2.49	105	3.85	-5.54
	121.1	4.76	-2.49	120	3.36	-6.03
	136.1	5.02	-2.24	135	2.18	-7.21
	151.1	4.79	-2.46	150	2.30	-7.09
REP 3	0	7.30	0.00	0	8.62	0.00
	16.1	5.46	-1.84	30	6.10	-2.53
	31.1	5.66	-1.64	45	4.18	-4.45
	46.1	4.74	-2.56	60	5.04	-3.58
	61.1	5.01	-2.29	75	3.48	-5.15
	76.1	4.79	-2.51	120	2.60	-6.02
	91.1	4.92	-2.38			
	106.1	5.14	-2.16			
	121.1	5.11	-2.19			
	136.1	4.94	-2.36			
	151.1	4.30	-3.00			

Table A.3 *Salmonella* inactivation data during isothermal treatment (80°C) for wheat meal (~0.4 a_w) using pre-fabrication and post-fabrication inoculation protocols.

	Pre-fabrication			Post-fabrication		
Rep	Time (min)	Log CFU/g	Log N/N_0	Time (min)	Log CFU/g	Log N/N_0
REP 1	0	8.70	0.00	0	8.59	0.00
	6	7.60	-1.10	15	7.46	-1.13
	12	6.81	-1.89	30	5.93	-2.66
	18	6.03	-2.67	45	6.01	-2.58
	24	5.99	-2.71	60	5.37	-3.22
	30	5.68	-3.02	75	4.41	-4.18
	36	5.44	-3.26	90	4.56	-4.03
	42	5.09	-3.61	105	3.82	-4.77
	48	5.13	-3.57			
REP 2	0	8.28	0.00	0	8.54	0.00
	6	7.41	-0.86	15	7.24	-1.29
	12	6.93	-1.34	30	5.48	-3.06
	18	6.18	-2.10	45	5.29	-3.25
	24	5.79	-2.49	60	4.80	-3.74
	30	5.42	-2.86	75	4.40	-4.14
	36	4.83	-3.45	90	3.48	-5.05
	42	4.56	-3.72	105	3.59	-4.95
	48	3.54	-4.73			
REP 3	0	8.50	0.00	0	8.45	0.00
	6	7.41	-1.08	15	7.03	-1.42
	12	6.93	-1.56	30	6.16	-2.29
	18	6.18	-2.32	45	6.33	-2.11
	24	5.79	-2.71	60	5.35	-3.09
	30	5.42	-3.08	75	4.24	-4.20
	36	4.83	-3.67	90	3.84	-4.61
	42	4.56	-3.94	120	3.69	-4.76
	48	3.54	-4.95			

Table A.4 *Salmonella* inactivation data during isothermal treatment (80°C) for wheat flour (~0.4 a_w) using pre-fabrication and post-fabrication inoculation protocols.

	Pre-fabrication			Post-fabrication		
Rep	Time (min)	Log CFU/g	Log N/N_0	Time (min)	Log CFU/g	Log N/N_0
REP 1	0	8.71	0.00	0	0	0.00
	6	7.61	-1.10	15	15	-1.65
	12	6.46	-2.25	30	30	-2.71
	18	6.51	-2.20	45	45	-2.90
	24	6.07	-2.64	60	60	-3.38
	30	5.04	-3.67	75	75	-3.93
	36	4.86	-3.84	90	90	-5.33
	42	4.28	-4.43	105	105	-6.37
	48	3.84	-4.87			
REP 2	0	8.53	0.00	0	0	0.00
	6	7.20	-1.33	15	15	-2.53
	12	5.90	-2.63	30	30	-2.69
	18	5.87	-2.67	45	45	-3.03
	24	5.48	-3.05	60	60	-4.63
	30	4.18	-4.36	75	75	-5.04
	36	3.00	-5.53	90	90	-4.71
	42	3.63	-4.90	105	105	-7.22
				120	120	-7.57
REP 3	0	8.54	0.00	0	0	0.00
	6	8.13	-0.41	15	15	-2.82
	12	6.90	-1.65	30	30	-4.19
	18	6.92	-1.62	45	45	-4.61
	24	5.92	-2.62	60	60	-5.86
	30	5.31	-3.23	75	75	-5.26
	36	4.82	-3.72	90	90	-5.98
	42	4.76	-3.78	105	105	-6.41
	48	4.12	-4.43			

Table A.5 *Salmonella* inactivation data during isothermal treatment (80°C) for date paste (~0.45 a_w) using pre-fabrication and post-fabrication inoculation protocols.

	Pre-fabrication			Post-fabrication		
Rep	Time (min)	Log CFU/g	Log N/N_0	Time (min)	Log CFU/g	Log N/N_0
REP 1	0	6.26	0.00	0	5.02	0.00
	20	6.70	0.44	20	4.45	-0.57
	40	6.03	-0.23	40	4.10	-0.93
	60	5.79	-0.46	60	3.19	-1.83
	80	5.75	-0.51	100	2.60	-2.42
	100	5.45	-0.81	120	2.81	-2.21
	120	6.00	-0.26	140	2.98	-2.05
	140	5.65	-0.61			
	180	5.71	-0.54			
REP 2	0	6.80	0.00	0	5.09	0.00
	20	7.00	0.21	20	4.09	-1.00
	40	6.95	0.15	40	4.08	-1.01
	60	7.11	0.31	60	5.63	0.54
	80	6.07	-0.73	80	4.89	-0.20
	100	6.23	-0.57	100	4.34	-0.75
	120	6.45	-0.34	120	3.53	-1.56
				140	3.54	-1.55
				160	3.03	-2.06
REP 3	0	7.12	0.00	0	4.90	0.00
	20	6.63	-0.48	20	5.25	0.35
	40	7.08	-0.03	40	3.41	-1.49
	60	6.79	-0.32	60	3.72	-1.18
	80	6.14	-0.98	80	4.94	0.04
	120	6.24	-0.88	100	3.35	-1.55
	140	6.38	-0.73	120	2.86	-2.04
	160	5.84	-1.28	140	1.90	-3.00
				160	2.23	-2.67
REP 4	0	6.71	0.00	0	4.69	0.00
	30	6.64	-0.06	20	4.77	0.08
	40	6.42	-0.28	40	4.90	0.21
	60	7.27	0.56	60	4.13	-0.56
	100	6.24	-0.46	80	4.20	-0.49
	120	5.77	-0.93	100	3.54	-1.15
	140	6.12	-0.59	120	4.77	0.09
	180	6.08	-0.63	140	2.15	-2.54

Appendix B. Survivor Data for the Long-Term Storage Experiments (Chapter 4)

This appendix presents *Salmonella* inactivation data for whole almonds (~0.45 a_w) during isothermal treatments (80°C) after 0, 7, 15, 27, 68, 70, and 103 weeks of storage at room temperature.

Table B.1 *Salmonella* inactivation data during isothermal treatment (80°C) for whole almonds after 0, 7, 15, 27, 68, 70, and 103 weeks of storage at room temperature.

Storage time (weeks)	Time (min)	Rep 1	Rep 2	Rep 3
0	0	7.80	7.96	8.06
	12	7.23	7.04	7.35
	24	5.59	7.29	7.19
	36	5.14	4.41	4.67
	48	6.01	5.63	-
	60	5.94	-	
	72	-	-	-
	84	-	4.63	3.59
	96	4.68	3.98	-
	108	-	-	3.83
7	0	7.78	7.32	7.62
	12	5.69	6.91	6.86
	24	4.60	5.45	5.17
	36	4.18	5.10	6.11
	48	4.16	5.84	4.91
	60	3.98	5.16	3.92
	72	4.21	-	-
15	0	7.02	7.61	7.03
	12	-	5.63	5.41
	24	6.01	5.11	5.83
	36	4.35	4.77	4.12
	48	4.37	4.64	-
	60	2.60	-	-
	72	4.18	-	-
	84	-	-	3.85

Data points were excluded due to *Salmonella* survival counts being lower than 25 colonies/plate.

Table B.1 (cont'd).

Storage time (weeks)	Time (min)	Rep 1	Rep 2	Rep 3
27	0	7.15	-	-
	10	6.74	6.47	-
	20	5.28	4.49	5.64
	30	4.02	4.33	4.70
	40	5.65	6.55	4.48
	50	4.02	3.82	-
	70	4.53	-	-
68	0	6.05	6.22	5.80
	6	5.08	5.56	5.68
	12	5.31	-	-
	18	4.95	5.40	4.00
	24	-	-	4.97
	30	-	4.26	-
	36	-	-	-
	42	-	3.87	-
	48	-	-	3.72
70	0	5.72	7.13	6.22
	6	4.94	4.56	6.72
	12	4.84	4.46	5.68
	18	6.27	3.59	5.87
	24	4.08	4.77	3.41
	30	-	4.01	3.58
	36	-	3.61	-
	42	-	2.85	-
	48	-	2.60	2.72
103	0	4.75	6.27	4.92
	5	3.29	3.62	4.01
	10	3.43	6.06	-
	15	5.28	3.43	3.94
	20	3.43	2.67	4.08
	25	4.84	4.91	-
	30	-	3.46	2.77
	35	-	3.45	2.31
	40	-	3.04	3.30

Data points were excluded due to *Salmonella* survival counts being lower than 25 colonies/plate.

Appendix C. Product Properties

This appendix includes the size distribution for almond meal, wheat meal, and wheat flour. Almond meal was analyzed using Microtrac Laser light scattering method, and the wheat meal and wheat flour were analyzed using the American Society of Agricultural and Biological Engineers (ASABE) standard S319.2 – method of determining and expressing fineness of feed materials by sieving.

Table C.1 Size distribution for wheat meal and wheat flour.

Wheat meal		Wheat flour	
Size range (mm)	Fraction (%Mass)	Size range (mm)	Fraction (%Mass)
> 0.814	34.4	> 0.814	6.6
0.814 – 0.420	23.8	0.814 – 0.420	26.1
0.420 – 0.250	10.7	0.420 – 0.250	14.0
0.250 – 0.177	7.5	0.250 – 0.177	13.0
0.177 – 0.149	6.2	0.177 – 0.149	24.5
0.149 – 0.125	1.2	0.149 – 0.125	5.8
< 0.125	16.2	< 0.125	10.0

Table C.2 Size distribution for almond meal.

Peaks summary	
Size (µm)	Fraction (%Vol)
871.9	40.0
227.8	35.2
9.62	24.8

Appendix D. Photographs of Experimental Work

Figure D.1 Example of almond kernels conditioning in equilibration chamber.

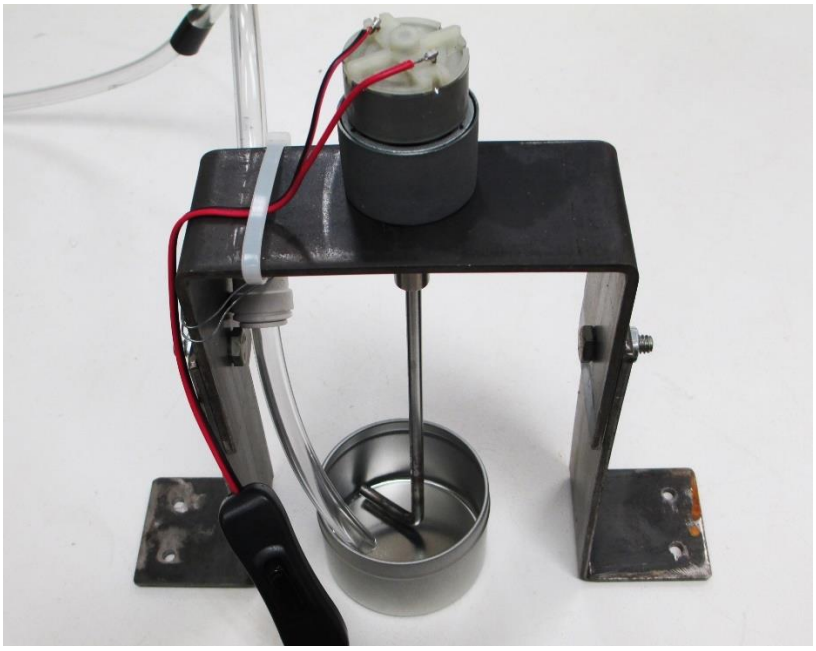

Figure D.2 Custom-designed stirrer using for equilibrating almond butter.

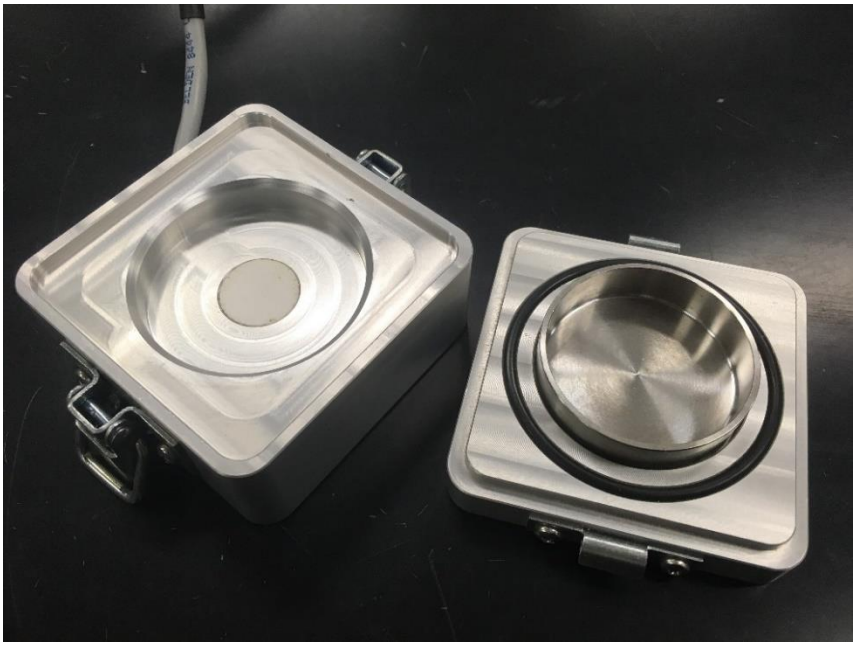

Figure D.3 Water activity meter for measuring a_w at high temperature.

Figure D.4 Example of almond products in plastic bag and aluminum test-cell for thermal inactivation studies.

Appendix E. Come-Up Time for Thermal Inactivation

This appendix shows the come-up time for thermal inactivation in this study, based on 6 replicates.

Table E.1 Come-up time (± standard deviation) for almond products.

Sample	80°C	85°C	90°C
Almond kernels	2.7 ± 0.4	3.1 ± 0.2	3.1 ± 0.3
Almond meal	2.0 ± 0.1	2.3 ± 0.2	2.4 ± 0.1
Almond butter	2.1 ± 0.1	2.1 ± 0.1	2.0 ± 0.2

Table E.2 Come-up time (± standard deviation) for wheat products.

Sample	80°C	85°C	90°C
Wheat kernels	0.8 ± 0.2	0.8 ± 0.1	0.8 ± 0.1
Wheat meal	1.6 ± 0.2	1.7 ± 0.3	1.6 ± 0.4
Wheat flour	1.3 ± 0.2	1.6 ± 0.2	2.1 ± 0.1

Table E.3 Come-up time (± standard deviation) for date products.

Sample	70°C	75°C	80°C
Date pieces	3.4 ± 0.3	3.1 ± 1.3	1.9 ± 0.3
Date paste	1.0 ± 0.1	1.1 ± 0.1	2.5 ± 0.1

Appendix F. Survivor Data for Water Activity, Product Structure, and Temperature Experiments (Chapter 5)

This appendix presents *Salmonella* inactivation data after heating in an isothermal water bath at three different temperatures (80, 85, and 90°C for wheat and almond products, and 70, 75, and 80°C for date products). Samples were equilibrated at three different water activities (0.25, 0.45, and 0.65 a_w) before performing the thermal experiments.

Table F.1 *Salmonella* inactivation data for almond kernels.

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.25	80	REP 1	0	7.70	0.00	0.258	3.02
			9	6.36	-1.35	0.258	3.02
			18	6.29	-1.41	0.258	3.02
			27	6.51	-1.19	0.258	3.02
			36	5.16	-2.54	0.258	3.02
			45	4.83	-2.87	0.258	3.02
			54	3.69	-4.02	0.258	3.02
			63	2.30	-5.40	0.258	3.02
			72	2.59	-5.11	0.258	3.02
		REP 2	0	7.55	0.00	0.265	3.25
			9	7.16	-0.39	0.265	3.25
			18	5.67	-1.88	0.265	3.25
			27	5.88	-1.67	0.265	3.25
			36	6.18	-1.37	0.265	3.25
			45	5.95	-1.60	0.265	3.25
			54	4.65	-2.90	0.265	3.25
			63	4.31	-3.24	0.265	3.25
			72	3.72	-3.83	0.265	3.25
		REP 3	0	7.09	0.00	0.243	3.03
			9	7.21	0.13	0.243	3.03
			18	6.18	-0.90	0.243	3.03
			27	5.37	-1.71	0.243	3.03
			36	5.85	-1.24	0.243	3.03
			45	3.97	-3.12	0.243	3.03
			54	4.29	-2.80	0.243	3.03
			63	4.75	-2.33	0.243	3.03
			72	3.62	-3.47	0.243	3.03

Table F.1 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.45	80	REP 1	0	7.80	0.00	0.462	3.95
			12	7.23	-0.57	0.462	3.95
			24	5.59	-2.21	0.462	3.95
			36	5.14	-2.66	0.462	3.95
			48	6.01	-1.79	0.462	3.95
			60	5.94	-1.86	0.462	3.95
			96	4.68	-3.12	0.462	3.95
			108	2.88	-4.92	0.462	3.95
		REP 2	0	7.96	0.00	0.449	3.58
			12	7.04	-0.92	0.449	3.58
			24	7.29	-0.67	0.449	3.58
			36	4.41	-3.56	0.449	3.58
			48	5.63	-2.34	0.449	3.58
			60	4.04	-3.92	0.449	3.58
			84	4.63	-3.34	0.449	3.58
			96	3.98	-3.99	0.449	3.58
			108	2.85	-5.12	0.449	3.58
		REP 3	0	8.06	0.00	0.446	3.96
			12	7.35	-0.71	0.446	3.96
			24	7.19	-0.87	0.446	3.96
			36	4.67	-3.39	0.446	3.96
			48	4.28	-3.78	0.446	3.96
			60	6.97	-1.09	0.446	3.96
			84	3.59	-4.47	0.446	3.96
			96	2.60	-5.46	0.446	3.96
			108	3.83	-4.23	0.446	3.96
0.65	80	REP 1	0	7.81	0.00	0.647	5.06
			6	6.82	-0.99	0.647	5.06
			12	6.91	-0.90	0.647	5.06
			18	6.15	-1.66	0.647	5.06
			24	5.95	-1.86	0.647	5.06
			30	4.42	-3.39	0.647	5.06
			36	3.16	-4.65	0.647	5.06
			42	2.98	-4.83	0.647	5.06
			48	4.25	-3.56	0.647	5.06
		REP 2	0	7.20	0.00	0.66	5.60
			6	6.97	-0.22	0.66	5.60
			12	5.82	-1.38	0.66	5.60
			18	4.66	-2.54	0.66	5.60
			24	3.46	-3.73	0.66	5.60
			30	4.05	-3.15	0.66	5.60
			36	3.23	-3.96	0.66	5.60

Table F.1 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.65	80	REP 2	42	2.10	-5.10	0.66	5.60
			48	3.79	-3.41	0.66	5.60
		REP 3	0	6.87	0.00	0.638	5.58
			6	5.44	-1.43	0.638	5.58
			12	5.75	-1.12	0.638	5.58
			18	5.34	-1.53	0.638	5.58
			24	3.92	-2.95	0.638	5.58
			30	4.06	-2.81	0.638	5.58
			36	3.64	-3.23	0.638	5.58
			42	4.47	-2.40	0.638	5.58
0.25	85	REP 1	0	7.61	0.00	0.258	3.02
			5	7.30	-0.31	0.258	3.02
			10	6.35	-1.26	0.258	3.02
			15	5.51	-2.10	0.258	3.02
			20	6.19	-1.42	0.258	3.02
			25	4.24	-3.37	0.258	3.02
			30	2.48	-5.12	0.258	3.02
			35	3.26	-4.35	0.258	3.02
			40	2.79	-4.82	0.258	3.02
		REP 2	0	6.82	0.00	0.265	3.25
			5	6.12	-0.71	0.265	3.25
			10	6.53	-0.29	0.265	3.25
			15	6.70	-0.12	0.265	3.25
			20	5.92	-0.90	0.265	3.25
			25	5.72	-1.11	0.265	3.25
			30	4.05	-2.77	0.265	3.25
			35	3.91	-2.92	0.265	3.25
			40	3.35	-3.47	0.265	3.25
		REP 3	0	7.17	0.00	0.234	3.03
			5	7.31	0.14	0.234	3.03
			10	5.87	-1.30	0.234	3.03
			15	5.97	-1.20	0.234	3.03
			20	5.39	-1.78	0.234	3.03
			25	5.37	-1.80	0.234	3.03
			30	4.77	-2.40	0.234	3.03
			35	4.11	-3.06	0.234	3.03
			40	4.67	-2.50	0.234	3.03
0.45	85	REP 1	0	7.96	0.00	0.428	4.32
			7	6.07	-1.89	0.428	4.32
			14	6.62	-1.34	0.428	4.32
			21	6.26	-1.70	0.428	4.32
			28	5.60	-2.36	0.428	4.32

Table F.1 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.45	85	REP 1	35	4.14	-3.82	0.428	4.32
			42	4.45	-3.50	0.428	4.32
			49	3.67	-4.29	0.428	4.32
		REP 2	0	7.80	0.00	0.454	3.65
			7	7.00	-0.79	0.454	3.65
			14	5.76	-2.03	0.454	3.65
			21	5.39	-2.41	0.454	3.65
			28	5.62	-2.17	0.454	3.65
			35	4.81	-2.98	0.454	3.65
			42	4.89	-2.90	0.454	3.65
			49	3.40	-4.40	0.454	3.65
			56	2.54	-5.25	0.454	3.65
		REP 3	0	8.09	0.00	0.44	3.92
			7	6.60	-1.50	0.44	3.92
			14	6.60	-1.50	0.44	3.92
			21	6.34	-1.75	0.44	3.92
			28	5.18	-2.92	0.44	3.92
			35	3.53	-4.56	0.44	3.92
0.65	85	REP 1	0	7.44	0.00	0.647	5.06
			1	6.97	-0.47	0.647	5.06
			2	6.54	-0.90	0.647	5.06
			3	5.96	-1.48	0.647	5.06
			4	6.30	-1.14	0.647	5.06
			5	6.14	-1.30	0.647	5.06
			6	4.43	-3.01	0.647	5.06
			7	5.66	-1.78	0.647	5.06
			8	6.35	-1.09	0.647	5.06
		REP 2	0	6.99	0.00	0.66	5.60
			1	7.06	0.07	0.66	5.60
			2	6.27	-0.71	0.66	5.60
			3	6.33	-0.65	0.66	5.60
			4	5.73	-1.25	0.66	5.60
			5	3.74	-3.25	0.66	5.60
			6	5.49	-1.50	0.66	5.60
			7	4.37	-2.62	0.66	5.60
		REP 3	0	6.37	0.00	0.638	5.58
			1	6.23	-0.15	0.638	5.58
			2	6.07	-0.31	0.638	5.58
			3	6.04	-0.34	0.638	5.58
			4	5.57	-0.81	0.638	5.58
			5	3.26	-3.12	0.638	5.58
			6	4.14	-2.24	0.638	5.58

Table F.1 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.65	85	REP 3	7	4.93	-1.44	0.638	5.58
			8	3.19	-3.18	0.638	5.58
0.25	90	REP 1	0	7.08	0.00	0.258	3.02
			3	6.27	-0.81	0.258	3.02
			6	5.42	-1.66	0.258	3.02
			9	4.70	-2.37	0.258	3.02
			12	2.54	-4.53	0.258	3.02
			15	3.20	-3.87	0.258	3.02
			18	2.95	-4.12	0.258	3.02
			21	3.68	-3.40	0.258	3.02
			24	3.18	-3.90	0.258	3.02
		REP 2	0	6.64	0.00	0.265	3.25
			3	6.56	-0.08	0.265	3.25
			6	5.35	-1.29	0.265	3.25
			9	3.94	-2.70	0.265	3.25
			12	3.65	-2.99	0.265	3.25
			15	4.37	-2.28	0.265	3.25
			18	3.90	-2.74	0.265	3.25
			24	2.51	-4.14	0.265	3.25
		REP 3	0	6.47	0.00	0.234	3.03
			3	6.60	0.13	0.234	3.03
			6	5.69	-0.78	0.234	3.03
			9	5.35	-1.12	0.234	3.03
			12	5.12	-1.35	0.234	3.03
			15	4.64	-1.83	0.234	3.03
			18	3.44	-3.03	0.234	3.03
			21	2.70	-3.77	0.234	3.03
			24	3.26	-3.21	0.234	3.03
0.45	90	REP 1	0	6.72	0.00	0.428	4.32
			2.5	7.04	0.31	0.428	4.32
			5	6.11	-0.62	0.428	4.32
			7.5	5.96	-0.77	0.428	4.32
			10	5.60	-1.12	0.428	4.32
			12.5	4.97	-1.75	0.428	4.32
			15	4.43	-2.29	0.428	4.32
			17.5	2.81	-3.91	0.428	4.32
			20	3.32	-3.40	0.428	4.32
		REP 2	0	6.85	0.00	0.454	3.65
			2.5	6.61	-0.23	0.454	3.65
			5	5.09	-1.76	0.454	3.65
			7.5	5.96	-0.77	0.428	4.32
			10	5.60	-1.12	0.428	4.32

Table F.1 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.45	90	REP 2	12.5	3.89	-2.96	0.454	3.65
			15	4.50	-2.35	0.454	3.65
			20	4.73	-2.11	0.454	3.65
		REP 3	0	7.50	0.00	0.44	3.93
			2.5	6.58	-0.92	0.44	3.93
			5	5.19	-2.31	0.44	3.93
			7.5	5.03	-2.47	0.44	3.93
			10	3.77	-3.72	0.44	3.93
			15	3.23	-4.27	0.44	3.93
			17.5	3.57	-3.93	0.44	3.93
			20	3.33	-4.17	0.44	3.93
0.65	90	REP 1	0	6.51	0.00	0.647	5.06
			0.5	6.70	0.19	0.647	5.06
			1	5.80	-0.71	0.647	5.06
			1.5	5.72	-0.79	0.647	5.06
			2	5.18	-1.33	0.647	5.06
			2.5	4.16	-2.35	0.647	5.06
			3	3.61	-2.90	0.647	5.06
			4	2.37	-4.14	0.647	5.06
		REP 2	0	4.81	0.00	0.66	5.60
			0.5	5.31	0.51	0.66	5.60
			1	4.71	-0.09	0.66	5.60
			1.5	4.49	-0.31	0.66	5.60
			2	3.48	-1.33	0.66	5.60
			2.5	3.18	-1.63	0.66	5.60
			3	3.70	-1.11	0.66	5.60
			4	2.30	-2.51	0.66	5.60
		REP 3	0	5.89	0.00	0.638	5.58
			0.5	6.25	0.36	0.638	5.58
			1	3.84	-2.06	0.638	5.58
			1.5	3.67	-2.22	0.638	5.58
			2	4.04	-1.86	0.638	5.58
			2.5	4.04	-1.86	0.638	5.58
			3	3.97	-1.93	0.638	5.58
			3.5	3.12	-2.78	0.638	5.58
			4	1.54	-4.35	0.638	5.58

Table F.2 *Salmonella* inactivation data for almond meal.

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.25	80	REP 1	0	8.02	0.00	0.266	2.00
			24	7.59	-0.43	0.266	2.00
			48	7.30	-0.72	0.266	2.00
			72	7.08	-0.93	0.266	2.00
			96	6.70	-1.31	0.266	2.00
			120	6.46	-1.55	0.266	2.00
			144	5.95	-2.07	0.266	2.00
			168	5.84	-2.18	0.266	2.00
			192	5.31	-2.70	0.266	2.00
		REP 2	0	8.00	0.00	0.257	1.75
			24	7.74	-0.26	0.257	1.75
			48	7.32	-0.68	0.257	1.75
			72	7.06	-0.94	0.257	1.75
			96	6.84	-1.16	0.257	1.75
			120	6.78	-1.22	0.257	1.75
			144	6.35	-1.65	0.257	1.75
			168	6.05	-1.96	0.257	1.75
			192	5.87	-2.13	0.257	1.75
		REP 3	0	8.11	0.00	0.242	2.86
			24	7.58	-0.53	0.242	2.86
			48	7.17	-0.94	0.242	2.86
			72	6.74	-1.37	0.242	2.86
			96	6.40	-1.71	0.242	2.86
			120	6.06	-2.04	0.242	2.86
			144	5.81	-2.30	0.242	2.86
			168	5.22	-2.88	0.242	2.86
			192	5.17	-2.94	0.242	2.86
0.45	80	REP 1	0	8.28	0.00	0.435	3.94
			22	7.49	-0.79	0.435	3.94
			44	7.16	-1.12	0.435	3.94
			66	6.76	-1.52	0.435	3.94
			88	6.30	-1.98	0.435	3.94
			110	6.18	-2.10	0.435	3.94
			132	5.70	-2.58	0.435	3.94
			154	5.26	-3.02	0.435	3.94
			176	5.15	-3.12	0.435	3.94
		REP 2	0	7.69	0.00	0.443	3.74
			22	7.39	-0.30	0.443	3.74
			44	6.70	-0.99	0.443	3.74
			66	6.31	-1.37	0.443	3.74
			88	5.70	-1.99	0.443	3.74
			110	5.21	-2.48	0.443	3.74

Table F.2 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.45	80	REP 2	132	4.89	-2.79	0.443	3.74
			154	4.20	-3.48	0.443	3.74
			176	3.93	-3.75	0.443	3.74
		REP 3	0	8.36	0.00	0.432	4.05
			44	7.06	-1.30	0.432	4.05
			66	6.74	-1.62	0.432	4.05
			88	6.00	-2.36	0.432	4.05
			110	5.21	-3.15	0.432	4.05
			132	4.62	-3.74	0.432	4.05
			154	5.20	-3.16	0.432	4.05
0.65	80	REP 1	0	7.87	0.00	0.640	6.06
			10	6.77	-1.09	0.640	6.06
			20	6.85	-1.02	0.640	6.06
			30	6.19	-1.68	0.640	6.06
			40	5.79	-2.08	0.640	6.06
			50	5.21	-2.66	0.640	6.06
			70	4.41	-3.45	0.640	6.06
			80	3.02	-4.85	0.640	6.06
		REP 2	0	7.99	0.00	0.639	6.06
			10	7.34	-0.65	0.639	5.84
			20	6.61	-1.38	0.639	5.84
			30	6.04	-1.95	0.639	5.84
			40	5.51	-2.49	0.639	5.84
			50	5.18	-2.81	0.639	5.84
			60	4.44	-3.55	0.639	5.84
			70	4.43	-3.56	0.639	5.84
			80	3.65	-4.34	0.639	5.84
		REP 3	0	7.99	0.00	0.639	5.44
			10	7.32	-0.67	0.639	5.44
			20	6.82	-1.17	0.639	5.44
			30	6.29	-1.71	0.639	5.44
			40	5.88	-2.12	0.639	5.44
			50	5.32	-2.68	0.639	5.44
			60	5.01	-2.99	0.639	5.44
			70	4.84	-3.15	0.639	5.44
			80	4.32	-3.67	0.639	5.44
0.25	85	REP 1	0	7.95	0.00	0.266	2.00
			19	7.26	-0.69	0.266	2.00
			38	6.70	-1.25	0.266	2.00
			57	6.35	-1.60	0.266	2.00
			76	5.98	-1.96	0.266	2.00
			95	5.36	-2.59	0.266	2.00

Table F.2 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.25	85	REP 1	114	5.13	-2.82	0.266	2.00
			133	4.91	-3.04	0.266	2.00
			152	4.01	-3.93	0.266	2.00
		REP 2	0	7.85	0.00	0.257	1.75
			19	7.78	-0.07	0.257	1.75
			38	6.97	-0.89	0.257	1.75
			57	6.60	-1.25	0.257	1.75
			76	6.11	-1.74	0.257	1.75
			95	5.88	-1.97	0.257	1.75
			114	5.38	-2.47	0.257	1.75
			133	5.30	-2.55	0.257	1.75
			152	4.94	-2.91	0.257	1.75
		REP 3	0	7.84	0.00	0.242	2.86
			19	7.28	-0.57	0.242	2.86
			57	5.94	-1.90	0.242	2.86
			76	5.48	-2.36	0.242	2.86
			95	5.01	-2.83	0.242	2.86
			114	4.77	-3.07	0.242	2.86
			133	4.11	-3.73	0.242	2.86
			152	3.74	-4.10	0.242	2.86
0.45	85	REP 1	0	8.15	0.00	0.435	3.94
			12	7.61	-0.53	0.435	3.94
			24	7.01	-1.13	0.435	3.94
			36	6.34	-1.80	0.435	3.94
			60	5.16	-2.98	0.435	3.94
			72	5.47	-2.68	0.435	3.94
			84	4.33	-3.82	0.435	3.94
			96	4.24	-3.91	0.435	3.94
		REP 2	0	7.72	0.00	0.443	3.74
			12	7.13	-0.59	0.443	3.74
			24	6.59	-1.13	0.443	3.74
			36	6.02	-1.70	0.443	3.74
			48	5.37	-2.35	0.443	3.74
			60	4.84	-2.88	0.443	3.74
			72	4.39	-3.33	0.443	3.74
			84	3.98	-3.74	0.443	3.74
			96	3.45	-4.27	0.443	3.74
0.45	85	REP 3	0	8.06	0.00	0.432	4.05
			12	7.30	-0.76	0.432	4.05
			24	5.30	-2.76	0.432	4.05
			36	5.79	-2.27	0.432	4.05
			48	5.81	-2.25	0.432	4.05

Table F.2 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.45	85	REP 3	60	3.57	-4.48	0.432	4.05
			84	4.11	-3.94	0.432	4.05
			96	3.78	-4.28	0.432	4.05
0.65	85	REP 1	0	8.00	0.00	0.639	5.84
			4	7.24	-0.75	0.639	5.84
			8	6.38	-1.62	0.639	5.84
			12	5.83	-2.17	0.639	5.84
			16	6.13	-1.86	0.639	5.84
			20	4.74	-3.26	0.639	5.84
			24	4.38	-3.62	0.639	5.84
			28	3.56	-4.44	0.639	5.84
			32	3.32	-4.68	0.639	5.84
		REP 2	0	7.74	0.00	0.639	5.44
			4	7.00	-0.74	0.639	5.44
			8	6.65	-1.09	0.639	5.44
			12	5.96	-1.78	0.639	5.44
			16	5.35	-2.39	0.639	5.44
			20	3.62	-4.12	0.639	5.44
			24	4.34	-3.40	0.639	5.44
			28	4.09	-3.65	0.639	5.44
			32	3.52	-4.22	0.639	5.44
		REP 3	0	7.51	0.00	0.644	5.72
			4	6.83	-0.68	0.644	5.72
			8	6.54	-0.97	0.644	5.72
			12	5.79	-1.71	0.644	5.72
			16	5.05	-2.46	0.644	5.72
			20	4.59	-2.91	0.644	5.72
			24	4.30	-3.20	0.644	5.72
			28	3.93	-3.58	0.644	5.72
			32	3.65	-3.85	0.644	5.72
0.25	90	REP 1	0	7.92	0.00	0.266	2.00
			12	6.98	-0.94	0.266	2.00
			24	6.29	-1.63	0.266	2.00
			36	5.72	-2.20	0.266	2.00
			48	4.97	-2.95	0.266	2.00
			60	4.66	-3.26	0.266	2.00
			72	3.96	-3.96	0.266	2.00
			84	3.49	-4.43	0.266	2.00
			96	3.31	-4.61	0.266	2.00

Table F.2 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.25	90	REP 2	0	7.85	0.00	0.257	1.75
			12	7.00	-0.85	0.257	1.75
			24	6.36	-1.50	0.257	1.75
			36	5.83	-2.02	0.257	1.75
			48	5.74	-2.11	0.257	1.75
			60	5.24	-2.61	0.257	1.75
			72	4.60	-3.25	0.257	1.75
			84	4.45	-3.40	0.257	1.75
			96	4.16	-3.69	0.257	1.75
		REP 3	0	7.92	0.00	0.242	2.86
			12	6.92	-1.01	0.242	2.86
			24	6.32	-1.60	0.242	2.86
			36	5.36	-2.56	0.242	2.86
			48	4.87	-3.06	0.242	2.86
			60	4.17	-3.75	0.242	2.86
			72	3.40	-4.53	0.242	2.86
			84	3.16	-4.76	0.242	2.86
			96	2.40	-5.53	0.242	2.86
0.45	90	REP 1	0	7.93	0.00	0.435	3.94
			5	7.22	-0.71	0.435	3.94
			10	6.92	-1.01	0.435	3.94
			15	5.32	-2.60	0.435	3.94
			20	5.82	-2.11	0.435	3.94
			25	4.50	-3.43	0.435	3.94
			30	4.46	-3.46	0.435	3.94
			35	4.52	-3.41	0.435	3.94
			40	4.48	-3.44	0.435	3.94
		REP 2	0	7.79	0.00	0.442	3.74
			5	6.78	-1.01	0.442	3.74
			10	6.10	-1.69	0.442	3.74
			15	5.66	-2.13	0.442	3.74
			20	5.09	-2.70	0.442	3.74
			25	4.73	-3.06	0.442	3.74
			30	4.23	-3.56	0.442	3.74
			35	4.00	-3.79	0.442	3.74
			40	3.37	-4.42	0.442	3.74
		REP 3	0	7.95	0.00	0.432	4.05
			5	7.03	-0.92	0.432	4.05
			10	6.71	-1.25	0.432	4.05
			15	6.19	-1.76	0.432	4.05
			20	5.60	-2.36	0.432	4.05
			25	5.53	-2.42	0.432	4.05

Table F.2 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.45	90	REP 3	30	4.38	-3.57	0.432	4.05
			35	4.04	-3.92	0.432	4.05
			40	3.28	-4.68	0.432	4.05
0.65	90	REP 1	0	7.15	0.00	0.639	5.84
			1	6.80	-0.35	0.639	5.84
			2	6.45	-0.70	0.639	5.84
			3	6.07	-1.08	0.639	5.84
			4	5.66	-1.49	0.639	5.84
			5	5.51	-1.64	0.639	5.84
			6	4.93	-2.22	0.639	5.84
			7	4.35	-2.80	0.639	5.84
			8	4.33	-2.82	0.639	5.84
		REP 2	0	7.40	0.00	0.639	5.44
			1	6.76	-0.64	0.639	5.44
			2	6.68	-0.72	0.639	5.44
			3	6.19	-1.21	0.639	5.44
			4	6.00	-1.40	0.639	5.44
			5	5.49	-1.91	0.639	5.44
			6	4.99	-2.41	0.639	5.44
			7	4.24	-3.16	0.639	5.44
			8	4.21	-3.19	0.639	5.44
		REP 3	0	7.17	0.00	0.644	5.72
			1	6.81	-0.36	0.644	5.72
			2	6.45	-0.72	0.644	5.72
			3	6.13	-1.04	0.644	5.72
			4	5.54	-1.62	0.644	5.72
			5	5.42	-1.75	0.644	5.72
			6	5.09	-2.08	0.644	5.72
			7	4.66	-2.51	0.644	5.72
			8	4.39	-2.78	0.644	5.72

Table F.3 *Salmonella* inactivation data for almond butter.

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.25	80	REP 1	0	8.49	0.00	0.243	2.98
			24	8.00	-0.49	0.243	2.98
			48	7.59	-0.90	0.243	2.98
			72	7.28	-1.21	0.243	2.98
			96	6.92	-1.57	0.243	2.98
			120	5.71	-2.78	0.243	2.98
			144	6.40	-2.09	0.243	2.98
			168	6.05	-2.44	0.243	2.98
			192	5.68	-2.81	0.243	2.98
		REP 2	0	8.26	0.00	0.260	2.90
			24	8.91	0.64	0.260	2.90
			48	7.81	-0.45	0.260	2.90
			72	7.50	-0.76	0.260	2.90
			96	7.38	-0.88	0.260	2.90
			120	6.94	-1.32	0.260	2.90
			144	5.97	-2.29	0.260	2.90
			168	6.25	-2.01	0.260	2.90
			192	5.61	-2.65	0.260	2.90
		REP 3	0	8.29	0.00	0.250	2.69
			24	7.84	-0.45	0.250	2.69
			48	7.09	-1.20	0.250	2.69
			72	7.05	-1.24	0.250	2.69
			96	6.55	-1.74	0.250	2.69
			120	6.14	-2.15	0.250	2.69
			144	5.93	-2.36	0.250	2.69
			168	5.41	-2.87	0.250	2.69
			192	4.34	-3.95	0.250	2.69
0.45	80	REP 1	0	7.71	0.00	0.428	4.35
			22	6.69	-1.02	0.428	4.35
			44	6.02	-1.69	0.428	4.35
			66	5.36	-2.35	0.428	4.35
			88	5.14	-2.58	0.428	4.35
			110	4.27	-3.44	0.428	4.35
			132	4.07	-3.64	0.428	4.35
			154	3.63	-4.08	0.428	4.35
			176	3.28	-4.43	0.428	4.35
		REP 2	0	7.27	0.00	0.443	4.46
			22	5.50	-1.77	0.443	4.46
			44	5.62	-1.65	0.443	4.46
			66	4.99	-2.29	0.443	4.46
			88	3.92	-3.36	0.443	4.46
			110	3.77	-3.50	0.443	4.46

Table F.3 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.45	80	REP 2	132	3.32	-3.95	0.443	4.46
			154	2.13	-5.14	0.443	4.46
		REP 3	0	7.46	0.00	0.437	4.18
			22	6.58	-0.88	0.437	4.18
			44	6.25	-1.21	0.437	4.18
			66	6.20	-1.26	0.437	4.18
			88	5.83	-1.63	0.437	4.18
			110	5.74	-1.72	0.437	4.18
			132	5.11	-2.35	0.437	4.18
			154	5.18	-2.28	0.437	4.18
			176	4.83	-2.63	0.437	4.18
0.65	80	REP 1	0	7.61	0.00	0.648	5.94
			8	6.90	-0.72	0.648	5.94
			16	6.27	-1.34	0.648	5.94
			24	5.78	-1.83	0.648	5.94
			32	5.11	-2.51	0.648	5.94
			40	4.63	-2.98	0.648	5.94
			48	4.33	-3.28	0.648	5.94
			56	3.87	-3.74	0.648	5.94
			64	3.96	-3.66	0.648	5.94
		REP 2	0	7.46	0.00	0.634	5.91
			8	6.62	-0.84	0.634	5.91
			16	5.98	-1.49	0.634	5.91
			24	5.45	-2.01	0.634	5.91
			32	4.57	-2.89	0.634	5.91
			40	3.98	-3.48	0.634	5.91
			48	3.36	-4.10	0.634	5.91
			56	2.24	-5.22	0.634	5.91
			64	2.36	-5.10	0.634	5.91
		REP 3	0	7.53	0.00	0.649	5.94
			8	6.83	-0.70	0.649	5.94
			16	6.12	-1.41	0.649	5.94
			24	5.50	-2.03	0.649	5.94
			32	4.96	-2.57	0.649	5.94
			40	4.16	-3.37	0.649	5.94
			48	3.90	-3.63	0.649	5.94
			56	3.23	-4.29	0.649	5.94
			64	2.60	-4.93	0.649	5.94
0.25	85	REP 1	0	8.65	0.00	0.234	3.04
			19	7.96	-0.68	0.234	3.04
			38	7.36	-1.29	0.234	3.04
			58	5.72	-2.92	0.234	3.04

Table F.3 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.25	85	REP 1	76	6.10	-2.55	0.234	3.04
			95	5.91	-2.74	0.234	3.04
			114	5.20	-3.45	0.234	3.04
			133	4.85	-3.79	0.234	3.04
			152	4.10	-4.55	0.234	3.04
		REP 2	0	8.46	0.00	0.249	2.98
			19	7.61	-0.85	0.249	2.98
			38	7.14	-1.32	0.249	2.98
			57	6.48	-1.99	0.249	2.98
			76	6.00	-2.46	0.249	2.98
			95	5.70	-2.76	0.249	2.98
			114	5.12	-3.34	0.249	2.98
			133	4.54	-3.92	0.249	2.98
			152	4.09	-4.37	0.249	2.98
		REP 3	0	8.09	0.00	0.25	2.69
			19	7.44	-0.65	0.25	2.69
			38	6.88	-1.21	0.25	2.69
			57	6.24	-1.84	0.25	2.69
			76	5.61	-2.48	0.25	2.69
			95	4.96	-3.12	0.25	2.69
			114	4.52	-3.57	0.25	2.69
			133	4.37	-3.71	0.25	2.69
			152	3.97	-4.12	0.25	2.69
0.45	85	REP 1	0	7.51	0.00	0.438	4.35
			12	6.27	-1.24	0.438	4.35
			24	5.71	-1.80	0.438	4.35
			36	5.02	-2.49	0.438	4.35
			48	3.80	-3.71	0.438	4.35
			60	3.54	-3.97	0.438	4.35
			72	3.44	-4.07	0.438	4.35
			84	2.85	-4.67	0.438	4.35
			96	3.27	-4.24	0.438	4.35
		REP 2	0	7.15	0.00	0.443	4.46
			12	6.20	-0.95	0.443	4.46
			24	5.37	-1.78	0.443	4.46
			36	4.84	-2.32	0.443	4.46
			48	4.07	-3.08	0.443	4.46
			60	3.90	-3.25	0.443	4.46
			72	3.04	-4.11	0.443	4.46
			84	2.65	-4.50	0.443	4.46
			96	2.08	-5.07	0.443	4.46

Table F.3 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.45	85	REP 3	0	7.34	0.00	0.437	4.18
			12	6.59	-0.76	0.437	4.18
			24	6.27	-1.08	0.437	4.18
			36	5.99	-1.35	0.437	4.18
			48	5.70	-1.64	0.437	4.18
			60	5.31	-2.03	0.437	4.18
			72	4.84	-2.50	0.437	4.18
			84	4.61	-2.73	0.437	4.18
			96	4.29	-3.05	0.437	4.18
0.65	85	REP 1	0	7.16	0.00	0.648	5.94
			3	6.19	-0.97	0.648	5.94
			6	5.61	-1.55	0.648	5.94
			9	5.86	-1.30	0.648	5.94
			12	5.49	-1.67	0.648	5.94
			15	4.29	-2.87	0.648	5.94
			18	3.72	-3.44	0.648	5.94
			21	3.01	-4.15	0.648	5.94
			24	2.45	-4.71	0.648	5.94
		REP 2	0	7.14	0.00	0.634	5.95
			3	6.16	-0.98	0.634	5.95
			6	5.48	-1.65	0.634	5.95
			9	4.70	-2.43	0.634	5.95
			12	3.85	-3.28	0.634	5.95
			15	3.28	-3.86	0.634	5.95
			18	3.57	-3.57	0.634	5.95
			21	2.24	-4.89	0.634	5.95
		REP 3	0	7.28	0.00	0.649	5.94
			3	6.30	-0.98	0.649	5.94
			6	5.53	-1.75	0.649	5.94
			9	4.98	-2.30	0.649	5.94
			12	4.36	-2.91	0.649	5.94
			15	3.71	-3.57	0.649	5.94
			18	2.86	-4.41	0.649	5.94
			21	1.78	-5.50	0.649	5.94
			24	1.65	-5.62	0.649	5.94
0.25	90	REP 1	0	8.51	0.00	0.234	3.04
			12	7.54	-0.97	0.234	3.04
			24	6.84	-1.67	0.234	3.04
			36	5.86	-2.65	0.234	3.04
			48	5.74	-2.77	0.234	3.04
			60	5.28	-3.23	0.234	3.04
			72	4.65	-3.86	0.234	3.04

Table F.3 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.25	90	REP 1	84	3.36	-5.15	0.234	3.04
			96	2.88	-5.63	0.234	3.04
		REP 2	0	8.22	0.00	0.249	2.98
			12	7.54	-0.69	0.249	2.98
			24	6.67	-1.55	0.249	2.98
			36	6.02	-2.20	0.249	2.98
			48	4.81	-3.41	0.249	2.98
			60	4.58	-3.64	0.249	2.98
			72	3.54	-4.68	0.249	2.98
			84	3.43	-4.79	0.249	2.98
			96	3.11	-5.11	0.249	2.98
		REP 3	0	7.82	0.00	0.250	2.69
			12	7.12	-0.70	0.250	2.69
			24	6.22	-1.60	0.250	2.69
			36	5.76	-2.06	0.250	2.69
			48	4.87	-2.95	0.250	2.69
			60	4.40	-3.42	0.250	2.69
			72	3.86	-3.96	0.250	2.69
			87	3.24	-4.58	0.250	2.69
			96	2.93	-4.89	0.250	2.69
0.45	90	REP 1	0	7.03	0.00	0.446	4.23
			4	6.21	-0.82	0.446	4.23
			8	5.59	-1.44	0.446	4.23
			12	4.92	-2.11	0.446	4.23
			16	4.41	-2.62	0.446	4.23
			20	3.85	-3.19	0.446	4.23
			24	3.24	-3.79	0.446	4.23
			28	2.94	-4.09	0.446	4.23
			32	2.28	-4.75	0.446	4.23
		REP 2	0	7.18	0.00	0.443	4.46
			4	6.31	-0.88	0.443	4.46
			8	5.72	-1.46	0.443	4.46
			12	5.58	-1.60	0.443	4.46
			16	4.75	-2.44	0.443	4.46
			20	4.32	-2.86	0.443	4.46
			24	3.71	-3.47	0.443	4.46
			28	3.29	-3.89	0.443	4.46
			32	2.31	-4.87	0.443	4.46
		REP 3	0	7.34	0.00	0.437	4.18
			4	6.59	-0.76	0.437	4.18
			8	6.27	-1.08	0.437	4.18
			12	5.99	-1.35	0.437	4.18

Table F.3 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.45	90	REP 3	16	5.70	-1.64	0.437	4.18
			20	5.31	-2.03	0.437	4.18
			24	4.84	-2.50	0.437	4.18
			28	4.61	-2.73	0.437	4.18
			32	4.29	-3.05	0.437	4.18
0.65	90	REP 1	0	6.92	0.00	0.648	5.94
			1	6.17	-0.75	0.648	5.94
			2	5.59	-1.33	0.648	5.94
			3	4.90	-2.02	0.648	5.94
			4	4.27	-2.65	0.648	5.94
			5	3.77	-3.14	0.648	5.94
			6	3.10	-3.82	0.648	5.94
			7	2.96	-3.96	0.648	5.94
			8	2.77	-4.15	0.648	5.94
		REP 2	0	6.78	0.00	0.655	5.95
			1	6.17	-0.61	0.655	5.95
			2	5.30	-1.48	0.655	5.95
			3	4.68	-2.10	0.655	5.95
			4	4.19	-2.59	0.655	5.95
			5	3.45	-3.33	0.655	5.95
			6	2.93	-3.85	0.655	5.95
			7	2.60	-4.18	0.655	5.95
			8	1.65	-5.12	0.655	5.95
		REP 3	0	6.70	0.00	0.649	5.94
			1	5.70	-1.00	0.649	5.94
			2	5.03	-1.67	0.649	5.94
			3	4.53	-2.17	0.649	5.94
			4	3.53	-3.18	0.649	5.94
			5	2.68	-4.02	0.649	5.94
			6	2.00	-4.70	0.649	5.94
			7	2.18	-4.53	0.649	5.94

Table F.4 *Salmonella* inactivation data for wheat kernels.

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.25	80	REP 1	0	7.87	0.00	0.256	7.99
			10	7.46	-0.40	0.256	7.99
			20	6.62	-1.25	0.256	7.99
			30	6.06	-1.81	0.256	7.99
			40	5.95	-1.92	0.256	7.99
			50	5.07	-2.80	0.256	7.99
			60	5.19	-2.68	0.256	7.99
			70	4.88	-2.99	0.256	7.99
			80	2.93	-4.93	0.256	7.99
		REP 2	0	8.84	0.00	0.253	7.99
			10	8.44	-0.41	0.253	7.99
			20	8.10	-0.75	0.253	7.99
			30	7.75	-1.10	0.253	7.99
			40	7.12	-1.72	0.253	7.99
			50	6.47	-2.37	0.253	7.99
			60	6.36	-2.49	0.253	7.99
			70	6.09	-2.75	0.253	7.99
			80	4.67	-4.18	0.253	7.99
		REP 3	0	9.08	0.00	0.253	8.12
			10	8.10	-0.97	0.253	8.12
			20	7.61	-1.47	0.253	8.12
			30	7.06	-2.01	0.253	8.12
			40	6.85	-2.22	0.253	8.12
			50	6.31	-2.77	0.253	8.12
			60	5.74	-3.33	0.253	8.12
			70	5.51	-3.57	0.253	8.12
			80	4.71	-4.37	0.253	8.12
0.45	80	REP 1	0	9.03	0.00	0.451	9.80
			5	8.43	-0.60	0.451	9.80
			10	8.31	-0.73	0.451	9.80
			15	8.03	-1.00	0.451	9.80
			20	7.54	-1.49	0.451	9.80
			25	7.25	-1.78	0.451	9.80
			30	6.33	-2.70	0.451	9.80
			35	6.27	-2.76	0.451	9.80
			40	6.40	-2.63	0.451	9.80
		REP 2	0	8.74	0.00	0.440	10.04
			5	7.99	-0.75	0.440	10.04
			10	7.31	-1.43	0.440	10.04
			15	7.37	-1.38	0.440	10.04
			20	6.77	-1.97	0.440	10.04
			25	6.12	-2.62	0.440	10.04

Table F.4 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.45	80	REP 2	30	5.07	-3.68	0.440	10.04
			35	4.96	-3.78	0.440	10.04
			40	4.18	-4.57	0.440	10.04
		REP 3	0	8.50	0.00	0.450	10.15
			5	8.07	-0.43	0.450	10.15
			10	7.57	-0.93	0.450	10.15
			15	6.86	-1.65	0.450	10.15
			20	6.32	-2.18	0.450	10.15
			25	5.82	-2.69	0.450	10.15
			30	5.40	-3.10	0.450	10.15
			35	4.74	-3.76	0.450	10.15
			40	4.10	-4.40	0.450	10.15
0.65	80	REP 1	0	8.62	0.00	0.651	11.73
			3	7.47	-1.15	0.651	11.73
			6	7.02	-1.60	0.651	11.73
			9	5.91	-2.71	0.651	11.73
			12	5.53	-3.08	0.651	11.73
			15	5.25	-3.37	0.651	11.73
			18	3.21	-5.41	0.651	11.73
			21	2.44	-6.17	0.651	11.73
			24	1.31	-7.31	0.651	11.73
		REP 2	0	8.74	0.00	0.655	12.83
			3	8.41	-0.32	0.655	12.83
			6	7.96	-0.77	0.655	12.83
			9	7.19	-1.55	0.655	12.83
			12	6.55	-2.18	0.655	12.83
			15	5.97	-2.76	0.655	12.83
			18	4.93	-3.80	0.655	12.83
			21	4.30	-4.44	0.655	12.83
			24	2.69	-6.04	0.655	12.83
		REP 3	0	8.83	0.00	0.648	12.06
			3	7.32	-1.51	0.648	12.06
			6	7.58	-1.26	0.648	12.06
			9	7.21	-1.62	0.648	12.06
			12	6.60	-2.23	0.648	12.06
			15	4.86	-3.97	0.648	12.06
			18	3.49	-5.34	0.648	12.06
			21	3.62	-5.21	0.648	12.06
			24	2.32	-6.51	0.648	12.06
0.25	85	REP 1	0	7.52	0.00	0.256	7.99
			6	7.05	-0.47	0.256	7.99
			12	6.19	-1.33	0.256	7.99

Table F.4 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.25	85	REP 1	18	5.99	-1.53	0.256	7.99
			24	5.54	-1.98	0.256	7.99
			30	4.46	-3.07	0.256	7.99
			36	4.34	-3.18	0.256	7.99
			42	4.55	-2.97	0.256	7.99
			48	2.71	-4.81	0.256	7.99
		REP 2	0	8.61	0.00	0.253	7.99
			6	8.14	-0.47	0.253	7.99
			12	7.12	-1.48	0.253	7.99
			18	6.95	-1.66	0.253	7.99
			24	5.92	-2.69	0.253	7.99
			30	5.54	-3.07	0.253	7.99
			36	4.78	-3.83	0.253	7.99
			42	4.60	-4.01	0.253	7.99
			48	3.61	-4.99	0.253	7.99
		REP 3	0	8.85	0.00	0.253	8.12
			6	7.92	-0.93	0.253	8.12
			12	7.23	-1.62	0.253	8.12
			18	6.17	-2.68	0.253	8.12
			24	5.64	-3.21	0.253	8.12
			30	5.51	-3.34	0.253	8.12
			36	4.66	-4.19	0.253	8.12
			42	5.03	-3.82	0.253	8.12
			48	3.53	-5.32	0.253	8.12
0.45	85	REP 1	0	8.45	0.00	0.451	9.80
			2	8.60	0.15	0.451	9.80
			4	7.79	-0.66	0.451	9.80
			6	7.38	-1.07	0.451	9.80
			8	7.23	-1.22	0.451	9.80
			10	6.14	-2.31	0.451	9.80
			12	5.94	-2.52	0.451	9.80
			14	5.02	-3.43	0.451	9.80
			16	4.10	-4.35	0.451	9.80
		REP 2	0	8.39	0.00	0.440	10.04
			2	8.05	-0.34	0.440	10.04
			4	7.41	-0.98	0.440	10.04
			6	6.97	-1.42	0.440	10.04
			8	6.53	-1.86	0.440	10.04
			10	5.34	-3.05	0.440	10.04
			12	5.00	-3.39	0.440	10.04
			14	4.33	-4.06	0.440	10.04
			16	2.98	-5.41	0.440	10.04

Table F.4 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.45	85	REP 3	0	8.51	0.00	0.450	10.15
			2	7.81	-0.70	0.450	10.15
			4	7.21	-1.31	0.450	10.15
			6	7.07	-1.44	0.450	10.15
			8	6.16	-2.35	0.450	10.15
			10	5.61	-2.90	0.450	10.15
			12	5.18	-3.33	0.450	10.15
			14	3.87	-4.64	0.450	10.15
			16	3.29	-5.22	0.450	10.15
0.65	85	REP 1	0	8.38	0.00	0.651	11.73
			1	7.87	-0.51	0.651	11.73
			2	7.28	-1.10	0.651	11.73
			3	5.93	-2.45	0.651	11.73
			4	5.67	-2.71	0.651	11.73
			5	4.78	-3.60	0.651	11.73
			6	4.45	-3.92	0.651	11.73
			7	3.06	-5.31	0.651	11.73
		REP 2	0	8.64	0.00	0.655	12.83
			1	8.30	-0.34	0.655	12.83
			2	7.92	-0.72	0.655	12.83
			3	7.31	-1.33	0.655	12.83
			4	6.08	-2.56	0.655	12.83
			5	6.09	-2.56	0.655	12.83
			6	4.72	-3.93	0.655	12.83
			7	4.29	-4.35	0.655	12.83
			8	2.68	-5.96	0.655	12.83
		REP 3	0	8.78	0.00	0.648	12.06
			1	8.19	-0.59	0.648	12.06
			2	7.31	-1.46	0.648	12.06
			3	6.83	-1.95	0.648	12.06
			4	6.09	-2.68	0.648	12.06
			5	4.38	-4.40	0.648	12.06
			6	4.04	-4.73	0.648	12.06
			7	4.11	-4.67	0.648	12.06
			8	2.46	-6.31	0.648	12.06
0.25	90	REP 1	0	7.64	0.00	0.256	7.99
			3	7.53	-0.11	0.256	7.99
			6	6.64	-1.00	0.256	7.99
			9	5.59	-2.05	0.256	7.99
			12	4.79	-2.85	0.256	7.99
			15	4.72	-2.92	0.256	7.99
			18	4.04	-3.60	0.256	7.99

Table F.4 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.25	90	REP 1	21	4.50	-3.14	0.256	7.99
		REP 2	0	8.85	0.00	0.253	7.99
			3	8.14	-0.71	0.253	7.99
			6	7.57	-1.28	0.253	7.99
			9	6.72	-2.13	0.253	7.99
			12	6.04	-2.81	0.253	7.99
			15	5.29	-3.56	0.253	7.99
			18	5.41	-3.44	0.253	7.99
			21	4.18	-4.67	0.253	7.99
			24	4.09	-4.76	0.253	7.99
		REP 3	0	8.70	0.00	0.253	8.12
			3	7.24	-1.47	0.253	8.12
			6	6.86	-1.84	0.253	8.12
			9	6.05	-2.65	0.253	8.12
			12	5.03	-3.67	0.253	8.12
			15	4.74	-3.96	0.253	8.12
			18	3.01	-5.69	0.253	8.12
			21	2.98	-5.73	0.253	8.12
0.45	90	REP 1	0.00	8.61	0.00	0.451	9.80
			0.75	8.21	-0.40	0.451	9.80
			1.50	7.79	-0.83	0.451	9.80
			2.25	7.53	-1.08	0.451	9.80
			3.00	6.55	-2.07	0.451	9.80
			3.75	5.94	-2.67	0.451	9.80
			4.50	5.36	-3.25	0.451	9.80
			5.25	4.90	-3.71	0.451	9.80
			6.00	3.59	-5.02	0.451	9.80
		REP 2	0.00	8.33	0.00	0.440	10.04
			0.75	7.48	-0.85	0.440	10.04
			1.50	7.31	-1.01	0.440	10.04
			2.25	6.54	-1.79	0.440	10.04
			3.00	6.08	-2.25	0.440	10.04
			3.75	4.89	-3.43	0.440	10.04
			4.50	4.60	-3.72	0.440	10.04
			5.25	3.61	-4.72	0.440	10.04
			6.00	3.53	-4.80	0.440	10.04
		REP 3	0.00	8.10	0.00	0.450	10.15
			0.75	7.91	-0.19	0.450	10.15
			1.50	7.13	-0.97	0.450	10.15
			2.25	6.67	-1.43	0.450	10.15
			3.00	6.02	-2.08	0.450	10.15
			3.75	4.99	-3.10	0.450	10.15

Table F.4 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.45	90	REP 3	4.50	3.94	-4.16	0.450	10.15
			5.25	3.48	-4.62	0.450	10.15
0.65	90	REP 1	0.00	8.06	0.00	0.651	11.73
			0.33	7.41	-0.66	0.651	11.73
			0.66	6.94	-1.13	0.651	11.73
			0.99	6.48	-1.58	0.651	11.73
			1.32	4.31	-3.75	0.651	11.73
			1.65	3.27	-4.79	0.651	11.73
			1.98	3.04	-5.02	0.651	11.73
			2.31	3.68	-4.38	0.651	11.73
			2.64	2.90	-5.17	0.651	11.73
		REP 2	0.00	7.98	0.00	0.655	12.83
			0.33	8.05	0.07	0.655	12.83
			0.66	7.45	-0.53	0.655	12.83
			0.99	5.99	-1.99	0.655	12.83
			1.32	5.80	-2.19	0.655	12.83
			1.65	5.51	-2.47	0.655	12.83
			1.98	4.58	-3.40	0.655	12.83
			2.31	3.39	-4.59	0.655	12.83
			2.64	2.97	-5.02	0.655	12.83
		REP 3	0.00	8.25	0.00	0.648	12.06
			0.33	7.79	-0.46	0.648	12.06
			0.66	7.23	-1.02	0.648	12.06
			0.99	5.65	-2.60	0.648	12.06
			1.32	5.35	-2.89	0.648	12.06
			1.65	4.98	-3.27	0.648	12.06
			1.98	4.41	-3.84	0.648	12.06
			2.31	3.67	-4.58	0.648	12.06
			2.64	3.47	-4.77	0.648	12.06

Table F.5 Salmonella inactivation data for wheat meal.

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.25	80	REP 1	0	8.58	0.00	0.254	8.00
			13	7.71	-0.87	0.254	8.00
			26	7.06	-1.52	0.254	8.00
			39	6.83	-1.75	0.254	8.00
			52	6.47	-2.12	0.254	8.00
			65	6.04	-2.55	0.254	8.00
			78	5.67	-2.91	0.254	8.00
			91	5.23	-3.35	0.254	8.00
			104	5.14	-3.44	0.254	8.00
		REP 2	0	8.21	0.00	0.253	8.45
			13	7.61	-0.60	0.253	8.45
			26	7.12	-1.09	0.253	8.45
			39	6.97	-1.24	0.253	8.45
			52	6.32	-1.89	0.253	8.45
			65	6.33	-1.88	0.253	8.45
			78	5.70	-2.51	0.253	8.45
			91	5.32	-2.89	0.253	8.45
			104	5.34	-2.87	0.253	8.45
		REP 3	0	8.44	0.00	0.253	8.38
			13	7.74	-0.71	0.253	8.38
			26	7.22	-1.22	0.253	8.38
			39	7.13	-1.31	0.253	8.38
			52	6.51	-1.93	0.253	8.38
			65	6.43	-2.01	0.253	8.38
			78	5.85	-2.59	0.253	8.38
			91	5.62	-2.83	0.253	8.38
			104	5.07	-3.37	0.253	8.38
0.45	80	REP 1	0	8.35	0.00	0.457	10.00
			8	7.47	-0.88	0.457	10.00
			16	6.84	-1.51	0.457	10.00
			24	6.65	-1.70	0.457	10.00
			32	6.12	-2.22	0.457	10.00
			40	5.87	-2.48	0.457	10.00
			48	4.77	-3.58	0.457	10.00
			56	4.49	-3.85	0.457	10.00
			64	3.98	-4.37	0.457	10.00
		REP 2	0	8.14	0.00	0.460	9.81
			8	6.62	-1.52	0.460	9.81
			16	6.12	-2.02	0.460	9.81
			24	6.22	-1.92	0.460	9.81
			32	4.29	-3.85	0.460	9.81
			40	4.07	-4.07	0.460	9.81

Table F.5 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.45	80	REP 2	48	3.94	-4.21	0.460	9.81
			56	3.26	-4.89	0.460	9.81
		REP 3	0	8.83	0.00	0.450	10.26
			8	6.03	-2.80	0.450	10.26
			16	6.15	-2.68	0.450	10.26
			24	5.16	-3.68	0.450	10.26
			32	4.85	-3.98	0.450	10.26
			40	4.17	-4.66	0.450	10.26
			48	3.10	-5.73	0.450	10.26
			56	3.46	-5.37	0.450	10.26
0.65	80	REP 1	0	8.06	0.00	0.650	11.94
			2	7.12	-0.95	0.650	11.94
			4	6.64	-1.42	0.650	11.94
			6	5.53	-2.53	0.650	11.94
			8	5.29	-2.78	0.650	11.94
			10	5.00	-3.07	0.650	11.94
			12	4.60	-3.47	0.650	11.94
			14	3.71	-4.36	0.650	11.94
			16	2.38	-5.68	0.650	11.94
		REP 2	0	8.12	0.00	0.650	12.17
			2	7.35	-0.77	0.650	12.17
			4	7.06	-1.05	0.650	12.17
			6	6.41	-1.70	0.650	12.17
			8	6.17	-1.95	0.650	12.17
			10	6.16	-1.96	0.650	12.17
			12	5.51	-2.61	0.650	12.17
			14	4.61	-3.51	0.650	12.17
			16	4.37	-3.74	0.650	12.17
		REP 3	0	8.17	0.00	0.653	12.56
			2	7.53	-0.64	0.653	12.56
			4	7.15	-1.02	0.653	12.56
			6	6.68	-1.49	0.653	12.56
			8	6.12	-2.05	0.653	12.56
			10	5.44	-2.73	0.653	12.56
			12	5.28	-2.89	0.653	12.56
			14	4.53	-3.64	0.653	12.56
			16	4.30	-3.87	0.653	12.56
0.25	85	REP 1	0	8.24	0.00	0.254	8.00
			8	7.20	-1.04	0.254	8.00
			16	6.78	-1.47	0.254	8.00
			24	6.06	-2.19	0.254	8.00
			32	6.12	-2.12	0.254	8.00

Table F.5 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.25	85	REP 1	40	5.01	-3.23	0.254	8.00
			48	5.03	-3.21	0.254	8.00
			56	5.39	-2.86	0.254	8.00
			64	3.71	-4.54	0.254	8.00
		REP 2	0	8.17	0.00	0.253	8.45
			8	7.39	-0.78	0.253	8.45
			16	7.07	-1.10	0.253	8.45
			24	6.71	-1.46	0.253	8.45
			32	6.19	-1.98	0.253	8.45
			40	6.02	-2.15	0.253	8.45
			48	5.73	-2.44	0.253	8.45
			56	5.37	-2.80	0.253	8.45
			64	5.44	-2.73	0.253	8.45
		REP 3	0	8.14	0.00	0.253	8.38
			8	7.67	-0.47	0.253	8.38
			16	6.85	-1.29	0.253	8.38
			24	5.68	-2.46	0.253	8.38
			32	5.85	-2.29	0.253	8.38
			40	5.42	-2.72	0.253	8.56
			48	4.84	-3.30	0.253	8.58
			56	4.25	-3.88	0.253	8.61
			64	3.98	-4.15	0.253	8.63
0.45	85	REP 1	0	8.29	0.00	0.457	10.00
			2.5	7.22	-1.07	0.457	10.00
			5	6.51	-1.78	0.457	10.00
			7.5	6.52	-1.77	0.457	10.00
			10	6.41	-1.88	0.457	10.00
			12.5	5.78	-2.51	0.457	10.00
			15	4.86	-3.43	0.457	10.00
			17.5	4.53	-3.76	0.457	10.00
			20	4.27	-4.02	0.457	10.00
		REP 2	0	8.19	0.00	0.450	9.83
			2.5	6.99	-1.20	0.450	9.83
			5	6.01	-2.18	0.450	9.83
			7.5	5.67	-2.52	0.450	9.83
			10	4.89	-3.30	0.450	9.83
			12.5	4.31	-3.88	0.450	9.83
			15	3.04	-5.15	0.450	9.83
			17.5	3.32	-4.87	0.450	9.83
0.45	85	REP 3	0	7.68	0.00	0.458	9.87
			2.5	6.65	-1.04	0.458	9.87
			5	6.07	-1.62	0.458	9.87

Table F.5 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.45	85	REP 3	7.5	4.16	-3.53	0.458	9.87
			10	4.60	-3.09	0.458	9.87
			12.5	4.08	-3.61	0.458	9.87
			15	3.09	-4.60	0.458	9.87
			17.5	2.74	-4.95	0.458	9.87
			20	3.04	-4.65	0.458	9.87
0.65	85	REP 1	0.00	7.54	0.00	0.634	10.97
			0.75	6.94	-0.60	0.634	10.97
			1.50	6.52	-1.02	0.634	10.97
			2.25	6.11	-1.43	0.634	10.97
			3.00	5.38	-2.16	0.634	10.97
			3.75	4.49	-3.05	0.634	10.97
			4.50	3.59	-3.95	0.634	10.97
			5.25	2.96	-4.58	0.634	10.97
			6.00	2.47	-5.07	0.634	10.97
		REP 2	0.00	7.78	0.00	0.650	12.17
			0.75	7.26	-0.52	0.650	12.17
			1.50	6.80	-0.98	0.650	12.17
			2.25	5.88	-1.91	0.650	12.17
			3.00	5.08	-2.70	0.650	12.17
			3.75	4.38	-3.40	0.650	12.17
			4.50	4.47	-3.32	0.650	12.17
			5.25	4.01	-3.78	0.650	12.17
			6.00	3.41	-4.38	0.650	12.17
		REP 3	0.00	7.64	0.00	0.653	12.56
			0.75	7.03	-0.61	0.653	12.56
			1.50	6.40	-1.24	0.653	12.56
			2.25	6.01	-1.63	0.653	12.56
			3.00	5.11	-2.53	0.653	12.56
			3.75	5.48	-2.16	0.653	12.56
			4.50	4.80	-2.84	0.653	12.56
			5.25	3.75	-3.89	0.653	12.56
			6.00	3.24	-4.40	0.653	12.56
0.25	90	REP 1	0	8.07	0.00	0.254	8.00
			3	6.85	-1.21	0.254	8.00
			6	6.24	-1.83	0.254	8.00
			9	5.99	-2.08	0.254	8.00
			12	5.12	-2.95	0.254	8.00
			15	4.48	-3.58	0.254	8.00
			18	3.76	-4.31	0.254	8.00
			21	3.78	-4.29	0.254	8.00
			24	3.33	-4.73	0.254	8.00

Table F.5 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.25	90	REP 2	0	8.09	0.00	0.253	8.45
			3	7.18	-0.91	0.253	8.45
			6	6.69	-1.40	0.253	8.45
			9	5.44	-2.65	0.253	8.45
			12	5.89	-2.19	0.253	8.45
			15	5.31	-2.77	0.253	8.45
			18	4.81	-3.28	0.253	8.45
			21	4.60	-3.48	0.253	8.45
			24	3.40	-4.68	0.253	8.45
		REP 3	0	8.06	0.00	0.253	8.38
			3	7.56	-0.50	0.253	8.38
			6	6.57	-1.49	0.253	8.38
			9	6.07	-1.99	0.253	8.38
			12	5.36	-2.70	0.253	8.38
			15	4.83	-3.23	0.253	8.38
			18	4.48	-3.58	0.253	8.38
			21	3.72	-4.34	0.253	8.38
			24	3.75	-4.31	0.253	8.38
0.45	90	REP 1	0	7.48	0.00	0.457	10.00
			0.5	7.04	-0.44	0.457	10.00
			1	6.48	-1.00	0.457	10.00
			1.5	6.47	-1.01	0.457	10.00
			2	5.80	-1.68	0.457	10.00
			2.5	5.51	-1.97	0.457	10.00
			3	5.12	-2.36	0.457	10.00
			3.5	4.37	-3.11	0.457	10.00
			4	4.06	-3.42	0.457	10.00
		REP 2	0	7.45	0.00	0.460	9.81
			0.5	7.96	0.51	0.460	9.81
			1	6.05	-1.40	0.460	9.81
			1.5	5.57	-1.88	0.460	9.81
			2	4.88	-2.57	0.460	9.81
			2.5	4.05	-3.40	0.460	9.81
			3	3.18	-4.27	0.460	9.81
			3.5	3.21	-4.24	0.460	9.81
			4	3.23	-4.22	0.460	9.81
		REP 3	0	6.71	0.00	0.458	9.87
			0.5	5.93	-0.78	0.458	9.87
			1	5.76	-0.94	0.458	9.87
			1.5	5.27	-1.43	0.458	9.87
			2	4.73	-1.97	0.458	9.87
			2.5	4.17	-2.54	0.458	9.87

Table F.5 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.45	90	REP 3	3	2.96	-3.75	0.458	9.87
0.65	90	REP 1	0.00	6.47	0.00	0.643	11.94
			0.17	5.82	-0.64	0.643	11.94
			0.33	5.10	-1.36	0.643	11.94
			0.50	3.96	-2.51	0.643	11.94
			0.67	5.09	-1.38	0.643	11.94
			0.84	3.39	-3.07	0.643	11.94
			1.00	4.05	-2.42	0.643	11.94
			1.17	2.97	-3.49	0.643	11.94
			1.34	2.18	-4.29	0.643	11.94
		REP 2	0.00	6.88	0.00	0.650	12.17
			0.17	6.36	-0.52	0.650	12.17
			0.33	5.72	-1.16	0.650	12.17
			0.50	5.78	-1.10	0.650	12.17
			0.67	4.87	-2.01	0.650	12.17
			0.84	5.27	-1.62	0.650	12.17
			1.00	5.12	-1.76	0.650	12.17
			1.17	5.14	-1.75	0.650	12.17
			1.34	3.54	-3.34	0.650	12.17
		REP 3	0.00	4.72	0.00	0.653	12.56
			0.17	6.34	1.61	0.653	12.56
			0.33	5.93	1.21	0.653	12.56
			0.50	6.26	1.54	0.653	12.56
			0.67	5.70	0.98	0.653	12.56
			0.84	5.04	0.32	0.653	12.56
			1.17	3.92	-0.80	0.653	12.56
			1.34	3.17	-1.55	0.653	12.56

Table F.6 *Salmonella* inactivation data for wheat flour.

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.25	80	REP 1	0	7.94	0.00	0.252	8.09
			16	7.51	-0.43	0.252	8.09
			32	7.07	-0.87	0.252	8.09
			48	6.62	-1.33	0.252	8.09
			64	6.64	-1.30	0.252	8.09
			80	6.05	-1.90	0.252	8.09
			96	5.33	-2.61	0.252	8.09
			112	5.48	-2.46	0.252	8.09
			128	5.06	-2.89	0.252	8.09
		REP 2	0	8.34	0.00	0.244	7.27
			16	7.49	-0.85	0.244	7.27
			32	7.04	-1.30	0.244	7.27
			48	6.58	-1.76	0.244	7.27
			64	6.47	-1.88	0.244	7.27
			80	6.03	-2.32	0.244	7.27
			96	5.98	-2.36	0.244	7.27
			112	5.51	-2.83	0.244	7.27
			128	5.07	-3.28	0.244	7.27
		REP 3	0	8.13	0.00	0.252	8.30
			16	7.29	-0.84	0.252	8.30
			32	7.05	-1.08	0.252	8.30
			48	5.68	-2.45	0.252	8.30
			64	5.64	-2.49	0.252	8.30
			80	5.43	-2.70	0.252	8.30
			96	5.31	-2.82	0.252	8.30
			112	2.60	-5.53	0.252	8.30
			128	3.96	-4.17	0.252	8.30
0.45	80	REP 1	0	8.44	0.00	0.450	10.00
			6	7.75	-0.69	0.450	10.00
			12	7.30	-1.14	0.450	10.00
			18	6.94	-1.50	0.450	10.00
			24	6.41	-2.03	0.450	10.00
			30	6.35	-2.08	0.450	10.00
			36	5.49	-2.95	0.450	10.00
			42	5.97	-2.46	0.450	10.00
			48	5.27	-3.17	0.450	10.00
		REP 2	0	8.27	0.00	0.459	10.31
			6	6.84	-1.43	0.459	10.31
			12	6.39	-1.88	0.459	10.31
			18	5.59	-2.68	0.459	10.31
			30	4.47	-3.81	0.459	10.31
			36	3.77	-4.50	0.459	10.31

Table F.6 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.45	80	REP 2	42	3.52	-4.75	0.459	10.31
		REP 3	0	8.51	0.00	0.465	10.22
			6	7.04	4.99	0.465	10.22
			12	6.86	3.52	0.465	10.22
			18	6.03	3.34	0.465	10.22
			24	5.35	2.52	0.465	10.22
			30	4.36	1.83	0.465	10.22
			36	3.78	0.84	0.465	10.22
			42	3.73	0.26	0.465	10.22
			48	3.89	0.21	0.465	10.22
0.65	80	REP 1	0	7.97	0.00	0.646	12.01
			2	7.02	-0.95	0.646	12.01
			4	6.40	-1.57	0.646	12.01
			6	6.39	-1.58	0.646	12.01
			8	5.04	-2.93	0.646	12.01
			10	4.30	-3.67	0.646	12.01
			12	3.46	-4.50	0.646	12.01
			14	3.21	-4.75	0.646	12.01
			16	2.62	-5.35	0.646	12.01
		REP 2	0	8.22	0.00	0.637	12.63
			2	7.45	-0.77	0.637	12.63
			4	6.54	-1.68	0.637	12.63
			6	6.13	-2.09	0.637	12.63
			8	5.73	-2.49	0.637	12.63
			10	4.88	-3.34	0.637	12.63
			12	4.59	-3.64	0.637	12.63
			14	4.16	-4.06	0.637	12.63
			16	3.24	-4.98	0.637	12.63
		REP 3	0	8.22	0.00	0.652	12.70
			2	7.37	-0.85	0.652	12.70
			4	6.70	-1.52	0.652	12.70
			6	6.24	-1.98	0.652	12.70
			8	5.44	-2.78	0.652	12.70
			10	5.41	-2.81	0.652	12.70
			12	4.35	-3.87	0.652	12.70
			14	4.20	-4.03	0.652	12.70
			16	3.39	-4.83	0.652	12.70
0.25	85	REP 1	0	8.17	0.00	0.245	7.73
			11	6.96	-1.22	0.245	7.73
			22	6.08	-2.09	0.245	7.73
			33	5.90	-2.28	0.245	7.73
			44	5.36	-2.81	0.245	7.73

Table F.6 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.25	85	REP 1	55	4.41	-3.76	0.245	7.73
			66	4.20	-3.97	0.245	7.73
			77	4.23	-3.94	0.245	7.73
		REP 2	0	8.25	0.00	0.244	7.27
			11	7.24	-1.01	0.244	7.27
			22	6.38	-1.87	0.244	7.27
			33	6.02	-2.23	0.244	7.27
			44	5.18	-3.07	0.244	7.27
			55	5.08	-3.17	0.244	7.27
			66	5.28	-2.97	0.244	7.27
			77	5.01	-3.24	0.244	7.27
			88	3.71	-4.54	0.244	7.27
		REP 3	0	7.95	0.00	0.252	8.30
			11	7.22	-0.73	0.252	8.30
			22	6.40	-1.55	0.252	8.30
			33	5.69	-2.26	0.252	8.30
			44	5.06	-2.89	0.252	8.30
			55	4.26	-3.69	0.252	8.30
			77	3.37	-4.58	0.252	8.30
0.45	85	REP 1	0	8.43	0.00	0.450	10.00
			1.5	7.97	-0.46	0.450	10.00
			3	7.36	-1.06	0.450	10.00
			4.5	7.03	-1.40	0.450	10.00
			6	7.24	-1.19	0.450	10.00
			7.5	6.24	-2.19	0.450	10.00
			9	6.31	-2.12	0.450	10.00
			10.5	5.93	-2.50	0.450	10.00
			12	6.14	-2.29	0.450	10.00
		REP 2	0	8.09	0.00	0.459	10.31
			1.5	8.09	0.00	0.459	10.31
			3	6.66	-1.43	0.459	10.31
			4.5	5.76	-2.33	0.459	10.31
			6	5.66	-2.43	0.459	10.31
			7.5	5.30	-2.79	0.459	10.31
			9	4.96	-3.13	0.459	10.31
			10.5	4.26	-3.83	0.459	10.22
			12	4.30	-3.79	0.459	10.22
		REP 3	0	7.56	0.00	0.465	10.22
			1.5	6.85	-0.72	0.465	10.22
			3	6.16	-1.40	0.465	10.22
			4.5	5.92	-1.64	0.465	10.22
			6	5.28	-2.28	0.465	10.22

Table F.6 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.45	85	REP 3	7.5	5.41	-2.16	0.465	10.22
			9	4.31	-3.25	0.465	10.22
			10.5	4.22	-3.34	0.465	10.22
			12	3.69	-3.87	0.465	10.22
0.65	85	REP 1	0	7.23	0.00	0.646	12.01
			0.5	6.92	-0.31	0.646	12.01
			1	6.72	-0.52	0.646	12.01
			1.5	5.97	-1.26	0.646	12.01
			2	5.38	-1.85	0.646	12.01
			2.5	4.84	-2.40	0.646	12.01
			3	4.75	-2.48	0.646	12.01
			3.5	4.07	-3.16	0.646	12.01
			4	3.37	-3.87	0.646	12.01
		REP 2	0	7.37	0.00	0.637	12.63
			0.5	7.07	-0.30	0.637	12.63
			1	6.95	-0.42	0.637	12.63
			1.5	6.33	-1.04	0.637	12.63
			2	5.82	-1.55	0.637	12.63
			2.5	5.05	-2.32	0.637	12.63
			3	5.48	-1.89	0.637	12.63
			3.5	5.02	-2.35	0.637	12.63
			4	3.37	-4.00	0.637	12.63
		REP 3	0	7.36	0.00	0.652	12.70
			0.5	7.07	-0.29	0.652	12.70
			1	6.58	-0.78	0.652	12.70
			1.5	6.30	-1.06	0.652	12.70
			2	6.29	-1.07	0.652	12.70
			2.5	5.74	-1.62	0.652	12.70
			3	5.48	-1.88	0.652	12.70
			3.5	4.29	-3.07	0.652	12.70
			4	4.29	-3.07	0.652	12.70
0.25	90	REP 1	0	7.48	0.00	0.252	8.09
			4	6.75	-0.73	0.252	8.09
			8	5.83	-1.65	0.252	8.09
			12	5.01	-2.47	0.252	8.09
			16	4.67	-2.81	0.252	8.09
			20	4.54	-2.94	0.252	8.09
			24	3.31	-4.16	0.252	8.09
			28	4.14	-3.34	0.252	8.09
		REP 2	0	7.96	0.00	0.244	7.27
			4	7.42	-0.55	0.244	7.27
			8	6.50	-1.46	0.244	7.27

Table F.6 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.25	90	REP 2	12	6.05	-1.92	0.244	7.27
			16	5.52	-2.44	0.244	7.27
			20	4.35	-3.61	0.244	7.27
			24	3.98	-3.98	0.244	7.27
			28	4.47	-3.49	0.244	7.27
			32	2.85	-5.11	0.244	7.27
		REP 3	0	8.08	0.00	0.250	7.33
			4	6.38	-1.69	0.250	7.33
			8	5.50	-2.57	0.250	7.33
			12	4.61	-3.46	0.250	7.33
			16	4.09	-3.99	0.250	7.33
			20	3.60	-4.48	0.250	7.33
			24	3.03	-5.05	0.250	7.33
			28	2.55	-5.53	0.250	7.33
			32	1.81	-6.27	0.250	7.33
0.45	90	REP 1	0.00	6.90	0.00	0.451	10.00
			0.37	6.41	-0.49	0.451	10.00
			0.73	6.08	-0.82	0.451	10.00
			1.10	5.99	-0.91	0.451	10.00
			1.47	4.74	-2.17	0.451	10.00
			1.84	5.18	-1.72	0.451	10.00
			2.20	4.71	-2.19	0.451	10.00
			2.57	5.01	-1.89	0.451	10.00
			2.94	4.17	-2.73	0.451	10.00
		REP 2	0.00	6.55	0.00	0.459	10.31
			0.37	7.26	0.71	0.459	10.31
			0.73	5.59	-0.96	0.459	10.31
			1.10	6.07	-0.48	0.459	10.31
			1.47	5.96	-0.59	0.459	10.31
			1.84	4.43	-2.12	0.459	10.31
			2.20	4.20	-2.35	0.459	10.31
			2.57	3.58	-2.97	0.459	10.31
		REP 3	0.00	6.23	0.00	0.454	10.46
			0.37	5.67	-0.56	0.454	10.46
			0.73	5.07	-1.16	0.454	10.46
			1.10	5.18	-1.05	0.454	10.46
			1.47	3.94	-2.29	0.454	10.46
			1.84	4.23	-2.00	0.454	10.46
			2.20	3.29	-2.94	0.454	10.46
			2.57	2.66	-3.57	0.454	10.46

Table F.6 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w	% MC, db
0.45	90	REP 3	2.94	2.75	-3.49	0.454	10.46
0.65	90	REP 1	0.00	4.07	0.00	0.649	12.82
			0.12	3.39	-0.69	0.649	12.82
			0.23	2.73	-1.35	0.649	12.82
			0.35	3.45	-0.63	0.649	12.82
			0.47	2.02	-2.05	0.649	12.82
			0.58	3.40	-0.67	0.649	12.82
			0.70	1.84	-2.23	0.649	12.82
			0.82	1.40	-2.68	0.649	12.82
		REP 2	0.00	5.12	0.00	0.637	12.63
			0.12	4.92	-0.19	0.637	12.63
			0.23	4.23	-0.88	0.637	12.63
			0.35	4.25	-0.87	0.637	12.63
			0.47	3.53	-1.59	0.637	12.63
			0.58	3.35	-1.77	0.637	12.63
			0.70	3.38	-1.73	0.637	12.63
			0.93	2.36	-2.76	0.637	12.63
		REP 3	0.00	5.30	0.00	0.652	12.70
			0.12	5.13	-0.17	0.652	12.70
			0.23	4.80	-0.50	0.652	12.70
			0.35	4.36	-0.95	0.652	12.70
			0.47	3.49	-1.81	0.652	12.70
			0.58	3.73	-1.58	0.652	12.70
			0.70	2.42	-2.88	0.652	12.70
			0.82	2.78	-2.52	0.652	12.70
			0.93	2.70	-2.60	0.652	12.70

Table F.7 *Salmonella* inactivation data for date pieces.

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w
0.25	70	REP 1	0	9.19	0.00	0.280
			3	9.41	0.22	0.280
			6	7.94	-1.25	0.280
			9	7.74	-1.45	0.280
			12	7.57	-1.62	0.280
			15	6.28	-2.90	0.280
			18	7.02	-2.17	0.280
			21	5.66	-3.53	0.280
			24	5.57	-3.62	0.280
		REP 2	0	8.53	0.00	0.265
			3	8.98	0.45	0.265
			6	7.95	-0.59	0.265
			9	7.94	-0.59	0.265
			12	7.89	-0.64	0.265
			15	6.83	-1.71	0.265
			18	7.46	-1.08	0.265
			21	6.99	-1.54	0.265
			24	4.89	-3.65	0.265
		REP 3	0	8.21	0.00	0.234
			3	8.74	0.54	0.234
			6	8.37	0.16	0.234
			9	7.70	-0.51	0.234
			12	8.08	-0.12	0.234
			15	7.81	-0.39	0.234
			18	7.26	-0.94	0.234
			21	7.14	-1.07	0.234
			24	6.41	-1.79	0.234
0.45	70	REP 1	0	9.09	0.00	0.442
			3	8.26	-0.84	0.442
			6	6.01	-3.08	0.442
			9	5.22	-3.87	0.442
			12	5.19	-3.90	0.442
			18	5.48	-3.61	0.442
			21	3.00	-6.09	0.442
		REP 2	0	8.05	0.00	0.453
			3	7.78	-0.27	0.453
			6	7.29	-0.77	0.453
			9	7.05	-1.00	0.453
			12	5.59	-2.46	0.453
			15	7.24	-0.81	0.453
			21	4.99	-3.06	0.453

Table F.7 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w
0.45	70	REP 3	0	8.02	0.00	0.442
			3	6.95	-1.06	0.442
			6	7.14	-0.88	0.442
			9	7.42	-0.60	0.442
			12	7.44	-0.58	0.442
			18	3.97	-4.04	0.442
0.65	70	REP 1	0	8.31	0.00	0.623
			3	7.13	-1.17	0.623
			6	7.60	-0.71	0.623
			9	7.40	-0.90	0.623
			12	6.83	-1.47	0.623
			15	6.98	-1.33	0.623
			21	3.13	-5.18	0.623
		REP 2	0	6.76	0.00	0.633
			3	8.64	1.88	0.633
			6	8.52	1.76	0.633
			12	7.40	0.64	0.633
			18	7.34	0.58	0.633
			21	3.82	-2.94	0.633
			24	4.91	-1.85	0.633
		REP 3	0	8.41	0.00	0.647
			3	7.30	-1.11	0.647
			6	6.73	-1.68	0.647
			9	6.26	-2.15	0.647
			12	3.91	-4.49	0.647
			18	3.22	-5.18	0.647
0.25	75	REP 1	0	7.64	0.00	0.280
			1.5	8.65	1.00	0.280
			4.5	6.79	-0.85	0.280
			6	6.89	-0.75	0.280
			7.5	6.43	-1.21	0.280
			9	6.21	-1.43	0.280
			12	5.34	-2.30	0.280
		REP 2	0	9.03	0.00	0.265
			1.5	8.49	-0.54	0.265
			3	8.17	-0.86	0.265
			4.5	7.66	-1.37	0.265
			6	7.39	-1.63	0.265
			7.5	6.36	-2.66	0.265
			9	5.75	-3.28	0.265
			10.5	4.38	-4.65	0.265
			12	3.80	-5.22	0.265

Table F.7 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w
0.25	75	REP 3	0	8.76	0.00	0.234
			1.5	8.39	-0.37	0.234
			3	8.16	-0.60	0.234
			4.5	7.80	-0.96	0.234
			6	6.73	-2.03	0.234
			7.5	6.33	-2.43	0.234
			9	7.29	-1.47	0.234
			10.5	4.85	-3.91	0.234
			12	4.76	-4.01	0.234
0.45	75	REP 1	0	6.28	0.00	0.442
			1.5	6.57	0.29	0.442
			3	7.34	1.06	0.442
			4.5	6.00	-0.28	0.442
			6	6.00	-0.28	0.442
			9	2.58	-3.70	0.442
		REP 2	0	7.23	0.00	0.453
			1.5	6.66	-0.57	0.453
			3	5.99	-1.25	0.453
			4.5	6.90	-0.33	0.453
			6	4.57	-2.66	0.453
			7.5	6.13	-1.10	0.453
			9	4.68	-2.55	0.453
			10.5	6.37	-0.86	0.453
		REP 3	0	8.87	0.00	0.442
			1.5	7.69	-1.18	0.442
			3	8.08	-0.79	0.442
			4.5	4.60	-4.27	0.442
			6	5.51	-3.36	0.442
			9	4.88	-3.99	0.442
			12	2.42	-6.45	0.442
0.65	75	REP 1	0	8.29	0.00	0.635
			1.5	8.21	-0.08	0.635
			3	7.64	-0.65	0.635
			4.5	6.72	-1.57	0.635
			6	5.81	-2.48	0.635
			7.5	5.83	-2.46	0.635
			10.5	4.50	-3.78	0.635
			12	5.42	-2.87	0.635
0.65	75	REP 2	0	7.42	0.00	0.644
			1.5	8.10	0.68	0.644
			3	6.27	-1.15	0.644
			4.5	4.55	-2.87	0.644

Table F.7 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w
0.65	75	REP 2	6	4.80	-2.62	0.644
			7.5	4.33	-3.09	0.644
			9	4.21	-3.21	0.644
			10.5	3.69	-3.73	0.644
			12	3.66	-3.76	0.644
		REP 3	0	7.32	0.00	0.656
			1.5	7.63	0.30	0.656
			3	6.37	-0.96	0.656
			4.5	6.85	-0.48	0.656
			6	4.58	-2.74	0.656
			7.5	2.99	-4.34	0.656
			9	3.86	-3.46	0.656
			0	9.05	0.00	0.280
			0.5	8.90	-0.14	0.280
0.25	80	REP 1	1	8.75	-0.30	0.280
			1.5	8.03	-1.02	0.280
			2	7.75	-1.30	0.280
			3	5.88	-3.17	0.280
			3.5	4.51	-4.53	0.280
			4	3.98	-5.06	0.280
			0	7.44	0.00	0.265
			0.5	6.51	-0.94	0.265
		REP 2	1	6.99	-0.45	0.265
			1.5	7.03	-0.41	0.265
			2	5.29	-2.16	0.265
			2.5	6.65	-0.79	0.265
			3	7.08	-0.36	0.265
			3.5	6.01	-1.44	0.265
			4	5.50	-1.95	0.265
			0	9.26	0.00	0.234
			0.5	9.03	-0.24	0.234
		REP 3	1	8.09	-1.17	0.234
			1.5	8.23	-1.03	0.234
			2	7.79	-1.47	0.234
			2.5	7.82	-1.44	0.234
			3	6.75	-2.51	0.234
			3.5	4.93	-4.33	0.234
			4	5.13	-4.14	0.234
0.45	80	REP 1	0	6.72	0.00	0.461
			0.5	5.74	-0.98	0.461
			1	5.06	-1.66	0.461
			1.5	4.74	-1.98	0.461

Table F.7 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w
0.45	80	REP 1	2	4.48	-2.24	0.461
			2.5	2.03	-4.69	0.461
			3	1.88	-4.85	0.461
		REP 2	0	8.61	0.00	0.453
			0.5	8.34	-0.27	0.453
			1	8.08	-0.52	0.453
			1.5	7.52	-1.09	0.453
			2	5.86	-2.74	0.453
			2.5	5.92	-2.68	0.453
			3	5.69	-2.91	0.453
			3.5	6.10	-2.51	0.453
			4	3.59	-5.02	0.453
		REP 3	0	8.58	0.00	0.455
			0.5	8.36	-0.22	0.455
			1	8.53	-0.04	0.455
			1.5	7.60	-0.98	0.455
			2	7.39	-1.19	0.455
			2.5	7.52	-1.06	0.455
			3	6.58	-1.99	0.455
			3.5	6.28	-2.29	0.455
			4	6.23	-2.35	0.455
0.65	80	REP 1	0	8.53	0.00	0.634
			0.5	6.96	-1.56	0.634
			1	7.09	-1.44	0.634
			1.5	7.08	-1.44	0.634
			2	5.22	-3.31	0.634
			2.5	4.60	-3.93	0.634
			3	4.12	-4.41	0.634
			3.5	3.43	-5.10	0.634
			4	3.52	-5.00	0.634
		REP 2	0	7.14	0.00	0.644
			0.5	7.09	-0.05	0.644
			1	6.91	-0.23	0.644
			1.5	5.30	-1.84	0.644
			2	3.16	-3.98	0.644
			2.5	3.57	-3.57	0.644
			4	3.95	-3.19	0.644

Table F.7 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w
0.65	80	REP 3	0	7.43	0.00	0.656
			0.5	7.15	-0.28	0.656
			1	6.71	-0.72	0.656
			1.5	5.32	-2.11	0.656
			2	5.48	-1.95	0.656
			2.5	6.47	-0.96	0.656
			3	6.60	-0.83	0.656
			3.5	5.14	-2.29	0.656
			4	2.11	-5.32	0.656

* Moisture content of date pieces was measured in the different batches.

** Moisture content of 0.25, 0.45, and 0.65 a_w date pieces was 10.17, 13.31, and 19.21 %MC, respectively.

Table F.8 Salmonella inactivation data for date paste.

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w
0.25	70	REP 1	0	8.27	0.00	0.244
			4	8.09	-0.18	0.244
			8	8.00	-0.27	0.244
			12	7.80	-0.47	0.244
			16	7.72	-0.55	0.244
			20	7.72	-0.55	0.244
			28	7.39	-0.87	0.244
			32	7.38	-0.88	0.244
		REP 2	0	8.05	0.00	0.263
			8	7.67	-0.38	0.263
			16	7.51	-0.54	0.263
			24	7.15	-0.90	0.263
			32	7.14	-0.90	0.263
			40	6.80	-1.25	0.263
			48	6.73	-1.31	0.263
			56	6.37	-1.68	0.263
			64	5.88	-2.17	0.263
		REP 3	0	7.69	0.00	0.255
			8	6.93	-0.76	0.255
			16	6.69	-1.00	0.255
			24	5.93	-1.77	0.255
			32	6.79	-0.91	0.255
			40	6.19	-1.50	0.255
			48	6.28	-1.41	0.255
			56	5.95	-1.74	0.255
			64	6.21	-1.49	0.255
0.45	70	REP 1	0	7.81	0.00	0.434
			3	7.95	0.14	0.434
			6	7.76	-0.05	0.434
			9	7.27	-0.55	0.434
			12	6.96	-0.85	0.434
			15	7.33	-0.48	0.434
			18	6.91	-0.90	0.434
			21	6.43	-1.39	0.434
		REP 2	0	7.95	0.00	0.449
			3	7.88	-0.07	0.449
			6	7.26	-0.69	0.449
			9	7.02	-0.93	0.449
			12	6.92	-1.03	0.449
			15	7.13	-0.82	0.449
			18	6.37	-1.58	0.449
			21	6.28	-1.67	0.449

Table F.8 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w
0.45	70	REP 3	0	8.15	0.00	0.445
			4	7.66	-0.49	0.445
			6	7.20	-0.95	0.445
			9	7.31	-0.84	0.445
			12	6.94	-1.21	0.445
			15	6.60	-1.55	0.445
			18	6.84	-1.31	0.445
			21	6.85	-1.31	0.445
			24	6.27	-1.89	0.445
0.65	70	REP 1	0	7.72	0.00	0.634
			2	7.14	-0.57	0.634
			4	6.97	-0.75	0.634
			6	6.53	-1.18	0.634
			8	6.48	-1.23	0.634
			10	6.02	-1.70	0.634
			12	4.59	-3.12	0.634
			14	5.95	-1.76	0.634
			16	4.73	-2.99	0.634
		REP 2	0	8.24	0.00	0.649
			2	7.78	-0.45	0.649
			4	7.57	-0.66	0.649
			6	6.34	-1.90	0.649
			8	6.07	-2.16	0.649
			10	6.80	-1.44	0.649
			12	5.66	-2.57	0.649
			14	5.75	-2.48	0.649
			16	5.27	-2.96	0.649
		REP 3	0	7.91	0.00	0.649
			2	7.22	-0.69	0.649
			4	7.37	-0.54	0.649
			6	6.16	-1.75	0.649
			8	6.30	-1.62	0.649
			10	4.48	-3.43	0.649
			12	5.07	-2.84	0.649
			14	4.48	-3.43	0.649
0.25	75	REP 1	0	8.31	0.00	0.254
			2	8.07	-0.24	0.254
			4	7.40	-0.91	0.254
			6	7.69	-0.63	0.254
			8	7.01	-1.30	0.254
			10	8.15	-0.16	0.254
			12	7.18	-1.13	0.254

Table F.8 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w
0.25	75	REP 1	14	6.65	-1.66	0.254
			16	6.39	-1.92	0.254
		REP 2	0	8.09	0.00	0.263
			4	7.52	-0.57	0.263
			8	7.22	-0.88	0.263
			12	7.00	-1.09	0.263
			16	6.76	-1.33	0.263
			20	6.51	-1.58	0.263
			24	6.85	-1.24	0.263
			28	6.88	-1.21	0.263
			32	5.64	-2.45	0.263
		REP 3	0	7.75	0.00	0.255
			4	6.89	-0.86	0.255
			8	6.68	-1.07	0.255
			12	6.48	-1.27	0.255
			16	6.02	-1.72	0.255
			20	6.63	-1.11	0.255
			24	6.09	-1.65	0.255
			28	6.18	-1.56	0.255
			32	5.67	-2.08	0.255
0.45	75	REP 1	0	7.73	0.00	0.447
			1.5	7.56	-0.18	0.447
			3	7.62	-0.11	0.447
			4.5	7.12	-0.61	0.447
			6	7.01	-0.72	0.447
			7.5	7.18	-0.56	0.447
			9	6.62	-1.11	0.447
			10.5	6.44	-1.29	0.447
			12	6.49	-1.24	0.447
		REP 2	0	7.45	0.00	0.461
			1.5	7.04	-0.42	0.461
			3	6.48	-0.98	0.461
			4.5	6.25	-1.20	0.461
			6	7.21	-0.24	0.461
			7.5	6.40	-1.06	0.461
			9	5.90	-1.55	0.461
			10.5	5.34	-2.12	0.461
			12	4.73	-2.72	0.461
		REP 3	0	7.72	0.00	0.449
			1.5	7.17	-0.56	0.449
			3	7.04	-0.68	0.449
			4.5	7.01	-0.72	0.449

Table F.8 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w
0.45	75	REP 3	6	6.56	-1.16	0.449
			7.5	6.75	-0.97	0.449
			9	6.74	-0.98	0.449
			10.5	6.32	-1.41	0.449
			12	6.39	-1.33	0.449
0.65	75	REP 1	0	8.03	0.00	0.641
			0.5	7.73	-0.30	0.641
			1	6.88	-1.15	0.641
			1.5	6.82	-1.21	0.641
			2	6.75	-1.28	0.641
			2.5	6.08	-1.95	0.641
			3	5.92	-2.11	0.641
			3.5	5.43	-2.60	0.641
			4	6.38	-1.65	0.641
		REP 2	0	7.57	0.00	0.634
			1	6.97	-0.61	0.634
			2	5.88	-1.69	0.634
			3	6.29	-1.29	0.634
			4	5.16	-2.41	0.634
			5	4.60	-2.98	0.634
			6	3.85	-3.73	0.634
			8	4.51	-3.07	0.634
		REP 3	0	8.11	0.00	0.649
			1	7.24	-0.87	0.649
			2	6.89	-1.22	0.649
			3	6.21	-1.90	0.649
			4	4.84	-3.27	0.649
			5	1.72	-6.39	0.649
			6	4.67	-3.44	0.649
			7	3.94	-4.17	0.649
			8	3.32	-4.78	0.649
0.25	80	REP 1	0.00	8.40	0.00	0.244
			0.67	8.11	-0.29	0.244
			1.33	8.02	-0.39	0.244
			2.00	8.15	-0.25	0.244
			2.67	8.13	-0.28	0.244
			3.33	7.89	-0.52	0.244
			4.67	7.56	-0.84	0.244
		REP 2	0.00	7.96	0.00	0.263
			1.33	7.68	-0.28	0.263
			2.67	7.25	-0.71	0.263
			4.00	6.95	-1.01	0.263

Table F.8 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w
0.25	80	REP 2	5.33	6.89	-1.06	0.263
			6.67	6.82	-1.14	0.263
			8.00	6.58	-1.38	0.263
			9.33	6.56	-1.40	0.263
			10.67	5.81	-2.15	0.263
		REP 3	0.00	7.41	0.00	0.255
			1.33	6.88	-0.52	0.255
			2.67	6.53	-0.88	0.255
			4.00	6.51	-0.90	0.255
			6.08	6.59	-0.82	0.255
			6.67	6.71	-0.70	0.255
			8.00	6.18	-1.23	0.255
			9.33	5.71	-1.69	0.255
			10.67	5.97	-1.43	0.255
0.45	80	REP 1	0.00	6.26	0.00	0.469
			0.33	6.70	0.44	0.469
			0.67	6.03	-0.23	0.469
			1.00	5.79	-0.46	0.469
			1.33	5.75	-0.51	0.469
			1.67	5.45	-0.81	0.469
			2.00	6.00	-0.26	0.469
			2.33	5.65	-0.61	0.469
			3.00	5.71	-0.54	0.469
		REP 2	0.00	7.12	0.00	0.450
			0.33	6.63	-0.48	0.450
			0.67	7.08	-0.03	0.450
			1.00	6.79	-0.32	0.450
			1.33	6.14	-0.98	0.450
			2.00	6.24	-0.88	0.450
			2.33	6.38	-0.73	0.450
			2.67	5.84	-1.28	0.450
		REP 3	0.00	7.71	0.00	0.432
			0.50	7.64	-0.06	0.432
			0.67	7.42	-0.28	0.432
			1.00	7.27	-0.44	0.432
			1.67	7.24	-0.46	0.432
			2.00	6.77	-0.93	0.432
			2.33	7.12	-0.59	0.432
			2.67	7.08	-0.63	0.432
0.65	80	REP 1	0.00	4.97	0.00	0.641
			0.33	4.11	-0.86	0.641
			0.67	4.56	-0.41	0.641

Table F.8 (cont'd).

Target a_w	Temp (°C)	Rep	Time (min)	Log CFU/g	Log N/N_0	Actual a_w
0.65	80	REP 1	1.00	4.25	-0.72	0.641
			1.67	3.71	-1.26	0.641
			2.67	1.95	-3.02	0.641
		REP 2	0.00	5.58	0.00	0.634
			0.33	4.42	-1.16	0.634
			1.00	4.75	-0.83	0.634
			1.67	1.00	-4.58	0.634
		REP 3	0.00	5.17	0.00	0.649
			0.33	4.97	-0.20	0.649
			0.67	2.40	-2.77	0.649
			1.67	3.15	-2.02	0.649

* Moisture content of date paste was measured in the different batches.

** Moisture content of 0.25, 0.45, and 0.65 a_w date paste was 12.02, 13.48, and 21.70 %MC, respectively.

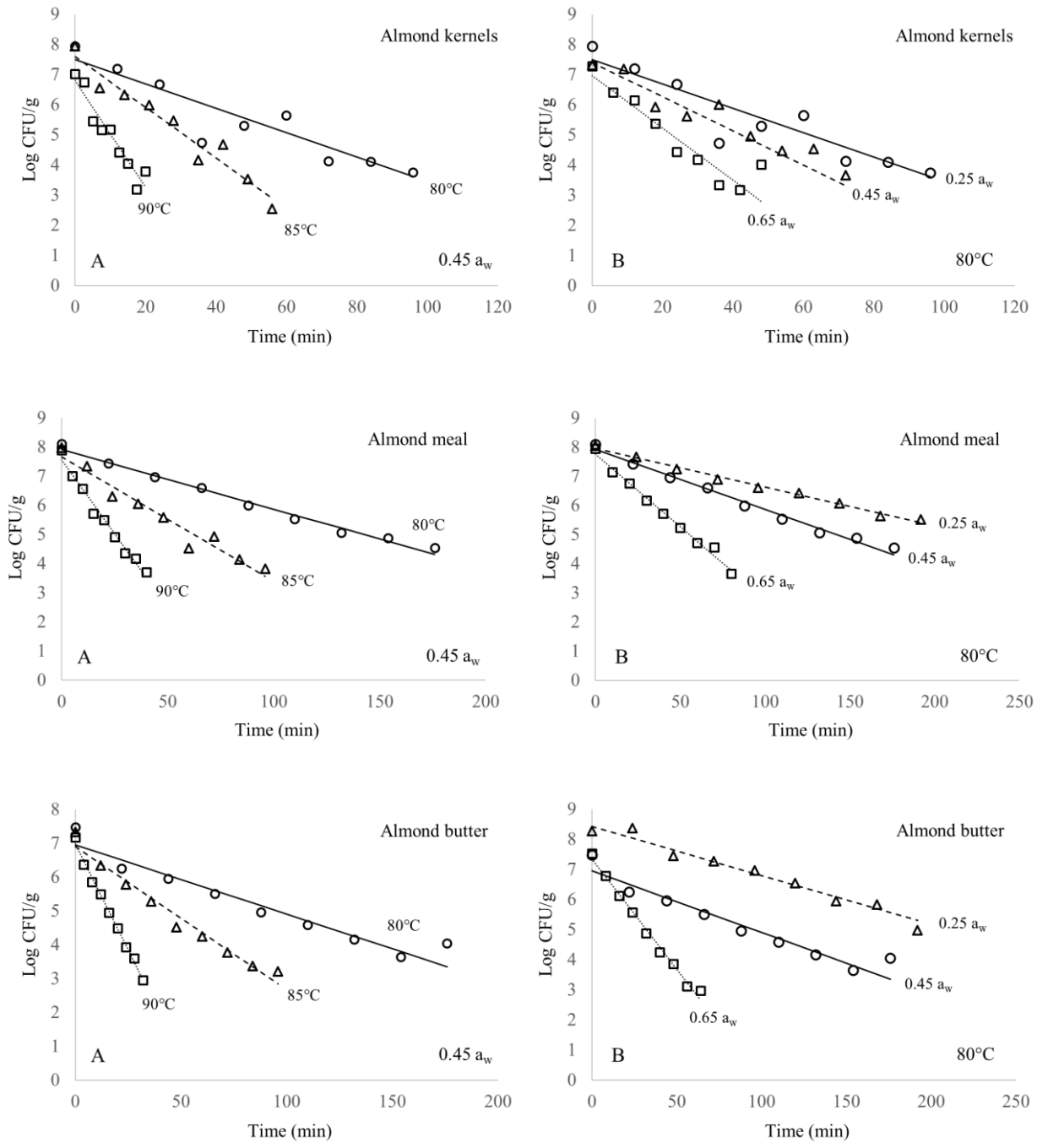

Figure F.1 Isothermal *Salmonella* survival curves and log-linear model fit for almond products at (A) constant a_w (0.45 a_w) with three different temperatures (80, 85, and 90°C), and (B) constant temperature (80°C) with three different a_w (0.25, 0.45, and 0.65 a_w).

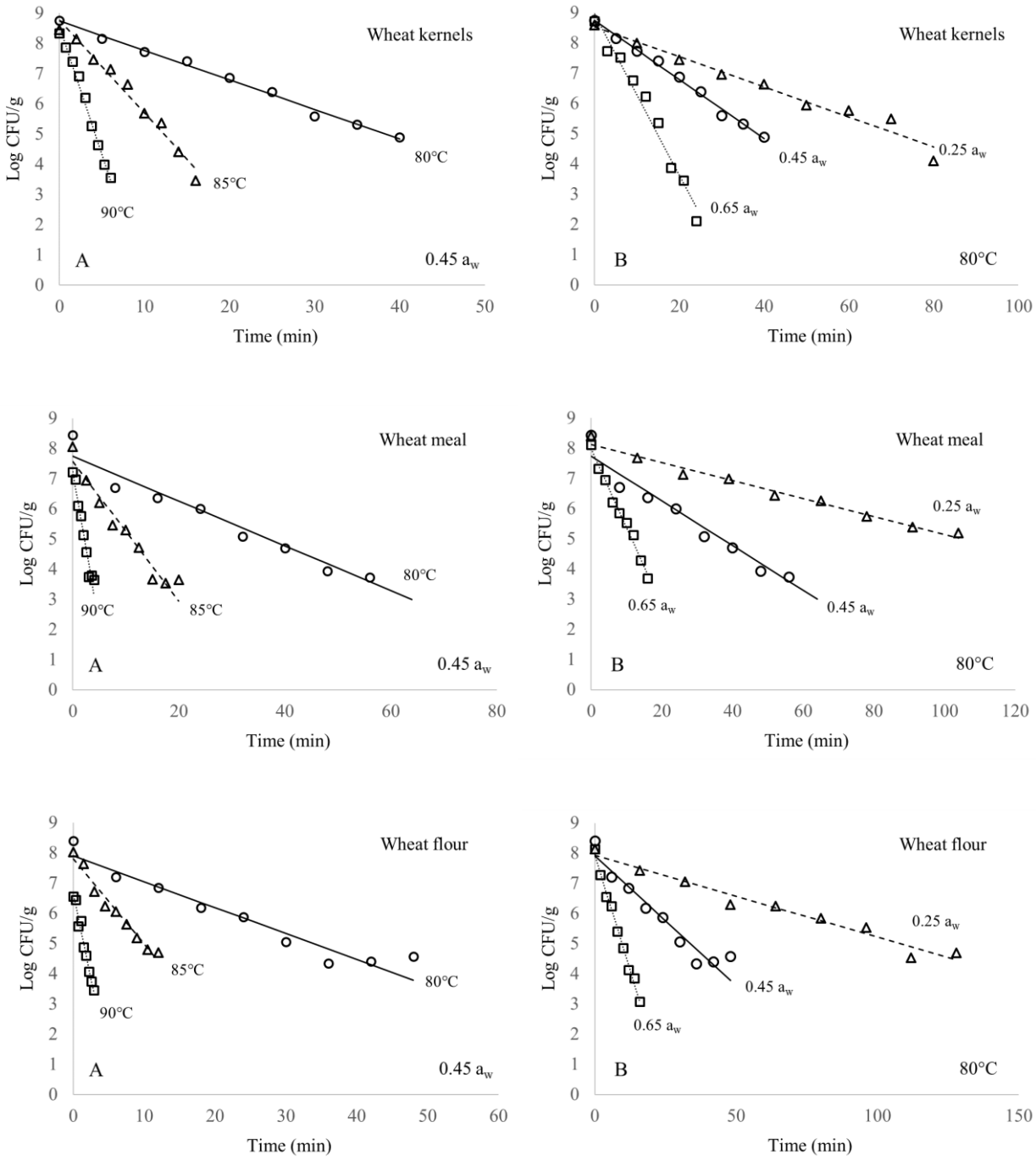

Figure F.2 Isothermal *Salmonella* survival curves and log-linear model fit for wheat products at (A) constant a_w (0.45 a_w) with three different temperatures (80, 85, and 90°C), and (B) constant temperature (80°C) with three different a_w (0.25, 0.45, and 0.65 a_w).

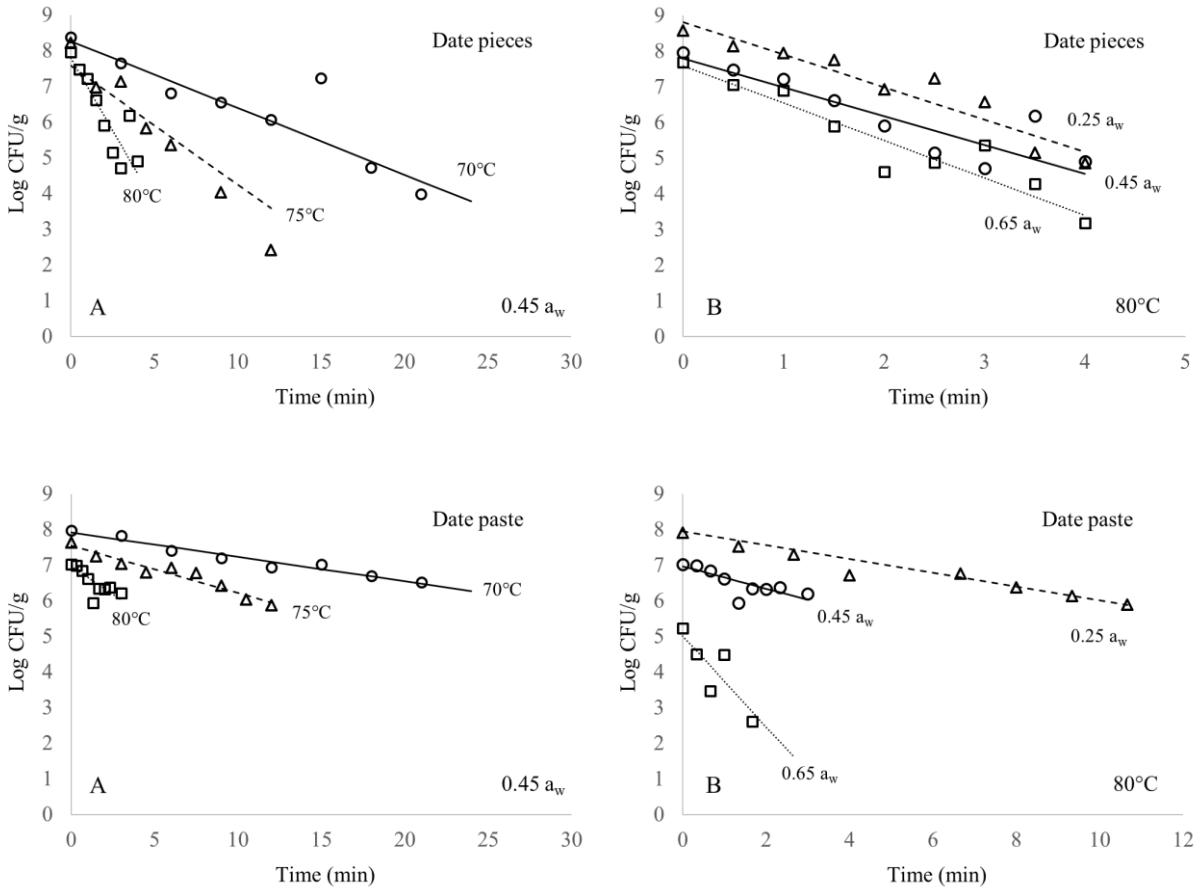

Figure F.3 Isothermal *Salmonella* survival curves and log-linear model fit for date products at (A) constant a_w (0.45 a_w) with three different temperatures (70, 75, and 80°C), and (B) constant temperature (80°C) with three different a_w (0.25, 0.45, and 0.65 a_w).

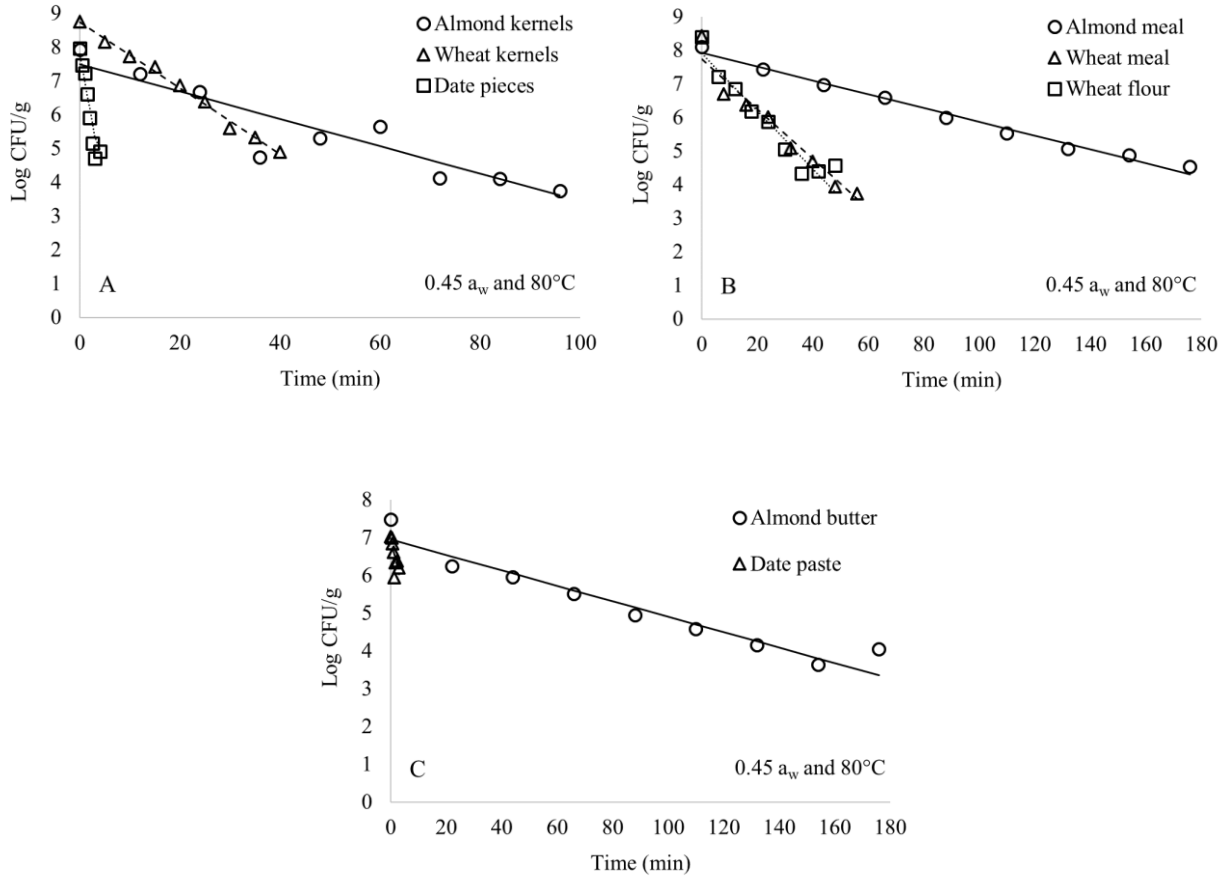

Figure F.4 Isothermal *Salmonella* survival curves and log-linear model fit of (A) almond kernels, wheat kernels, and date pieces, (B) almond meal, wheat meal, and wheat flour, and (C) almond butter and date paste at constant a_w (0.45 a_w) and temperature (80°C).

Appendix G. Matlab Codes for the GLM Regression

This appendix shows the example of MATLAB code used to fit the generalized linear model for almond products.

```
%% Import data, format for GLM
data=xlsread('data.xlsx');

y=data(:,2);         %Log N is Response

time=data(:,1);      %X1
x1=time;

temp=data(:,3);      %X2
x2=temp;

aw=data(:,4);        %X3
x3=aw;

structure=data(:,5);%X4
x4=structure;

%Interaction effects
x5=x1.*x2; %time*temp
x6=x1.*x3; %time*aw
x7=x1.*x4; %time*structure
x8=x2.*x3; %temp*aw
x9=x2.*x4; %temp*structure
x10=x3.*x4; %aw*structure

x11=x1.*x2.*x3; %time*temp*aw
x12=x1.*x2.*x4; %time*temp*structure
x13=x1.*x3.*x4; %time*aw*structure
x14=x2.*x3.*x4; %temp*aw*structure

X=[x1 x2 x3 x4 x5 x6 x7 x8 x9 x10 x11 x12 x13 x14];

%% GLM

mdl = GeneralizedLinearModel.fit(X,y)
```

Appendix H. Matlab Codes for Model Fitting

This appendix shows the example of MATLAB code used to fit log-linear, Weibull, and secondary models for almond kernels.

Example of the log-linear model fitting for almond kernels inactivation (0.25 a_w, 80°C)

```
% data=xlsread('input data_arrange.xlsx','almond kernel');
t=data(:,1); %time (min)
logn=data(:,2); %log N (CFU/ml)
temp=data(:,3); %heating temperature (c)
aw=data(:,4); %aw

%0.25aw and 80c
t_25_80=t(1:27); logn_25_80=logn(1:27); temp_25_80=temp(1:27);
aw_25_80=aw(1:27);

%Log-linear Model: log N(t) = -t/D + log N0

%0.25 80C
beta0=[5 7];  % beta0= [initial D, initial logN0];
fname=@nonlinearDC;
[beta,resids,J,COVB,mse] =
nlinfit(t_25_80,logn_25_80,fname,beta0);
D_25_80=beta;
ci = nlparci(beta,resids,'jacobian',J);
rmse=sqrt(mse);

% AIC
n=length(logn_25_80);
K=3;
logn_25_80_es = (-t_25_80/beta(1))+ beta(2);
residue = (logn_25_80-logn_25_80_es);
ss = sum(residue.^2);
AIC=n*log(ss/n)+2*K+2*K*(K+1)/(n-K-1);

result1 = [beta(1) ci(1) ci(3) n rmse AIC];
result2 = [beta(2) ci(2) ci(4)];

disp('0.25 almond kernels at 80C');
disp('   D-VALUE     CIL        CIU        n        RMSE
AIC');
disp(result1);
disp('    Log N0     CIL       CIU');
disp(result2);
```

```
function y = nonlinearD(beta,t)
    y= -t/beta(1) + beta(2);
end
```

Example of the Weibull model fitting for almond kernels inactivation (0.25 a_w, 80°C).

```
% data=xlsread('input data_arrange.xlsx','almond kernel');
t=data(:,1); %time (min)
logn=data(:,2); %log N (CFU/ml)
temp=data(:,3); %heating temperature (c)
aw=data(:,4); %aw

%0.25aw and 80c
t_25_80=t(1:27); logn_25_80=logn(1:27); temp_25_80=temp(1:27);
aw_25_80=aw(1:27);

%Weibull Model: log N(t) = -(t/delta)^p(shape parameter) + log
N0

%0.25 80C
beta0=[10 0.7 7];  % beta0= [initial D, initial logN0];
fname=@WeibullDC;
[beta,resids,J,COVB,mse] =
nlinfit(t_25_80,logn_25_80,fname,beta0);
D_25_80=beta;
ci = nlparci(beta,resids,'jacobian',J);
rmse=sqrt(mse);

n=length(logn_25_80);
K=4;
ss = sum(resids.^2,'omitnan');
AIC=n*log(ss/n)+2*K+2*K*(K+1)/(n-K-1);

%Estimated 1 log reduction
ts=linspace(min(t_25_80),max(t_25_80),1000);
[ypred, delta] =
nlpredci(fname,ts,beta,resids,J,0.05,'on','curve'); %confidence
band for regression line
CBu=ypred+delta;

log_require=beta(3)-1; %-1 is a log reduction required
xinterp=interp1(ypred, ts, log_require);
Estimated_log_reduction = xinterp;

CIU = interp1(CBu, ts, log_require); % c103 for CI at a log
reduction required
```

```
Upper_CI_for_yobs = CIU;

SE= (CIU-xinterp)/1.96;

result1 = [beta(1) ci(1) ci(4) n rmse AIC];
result2 = [beta(2) ci(2) ci(5)];
result3 = [beta(3) ci(3) ci(6)];

disp('0.25 almond kernels at 80C');
disp('    Delta      CIL       CIU        n        RMSE
AIC');
disp(result1);
disp('      P        CIL       CIU');
disp(result2);
disp('    Log N0     CIL       CIU');
disp(result3);

function y = WeibullDC(beta,t)
    y=(-1.*(t./beta(1)).^beta(2))+beta(3);
end
```

Example of the secondary model fitting for almond kernels inactivation.

```
clear;
clc;
data=xlsread('Normalization data.xlsx','almond kernel');
x=data(:,1); %time (min)
yobs=data(:,2); %log N (CFU/ml)
temp=data(:,3); %heating temperature (c)
aw=data(:,4); %actual aw
A =[x temp aw];

%% Secondary model
% log D = log D ref -((T-Tref)/ZT) - ((aw-awref)/Zaw)
% Tref and aw ref were estimated

beta0(1)=1; %log D ref
beta0(2)=15; %ZT
beta0(3)=1; %Zaw

%% nlinfit for secondary model
fnameINV=@DZ_fix;
[beta,resids,J,COVB,mse] = nlinfit(A,yobs,fnameINV,beta0);
beta
ss=resids'*resids;
n=length(x);
p=length(beta);
```

```
rmse=sqrt(mse)
condX=cond(J);
detXTX=det(J'*J);

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

%% R is the correlation matrix for the parameters, sigma is the
standard error vector
[R,sigma]=corrcov(COVB)
SS=resids'*resids
relstderr=sigma./beta
corrdz=R(2,1); %correlation between Dr and zT


%% AIC
n=length(yobs);
K=4;
ss = sum(resids.^2,'omitnan');
AICc=n*log(ss/n)+2*K+2*K*(K+1)/(n-K-1)


function logn = DZ_fix(beta,X)
% This function represents the secondary model
t=X(:,1);
temp =X(:,2);
aw=X(:,3);

%Tref and awref were optimized with smallest correlation between
%parameters

Tref = 81.4;
awref = 0.451;

Dvalue = beta(1)-((temp-Tref)./beta(2))-((aw-awref)./beta(3));
D=10.^(Dvalue);
logn=-t./D;
end
```

Appendix I. *Salmonella* Population Reductions during Thermal Come-Up Time (Chapter 5)

This appendix shows the reduction of *Salmonella* populations after samples were thermally treated and until the products reached the target temperature.

Table I.1 *Salmonella* population (± standard deviation) reduction during the thermal come-up time for almond products.

Products		*Salmonella* population (log CFU/g)		
		80°C	85°C	90°C
Almond kernels	0.25 a_w	0.98 ± 0.26	1.30 ± 0.19	1.74 ± 0.13
	0.45 a_w	0.60 ± 0.16	0.42 ± 0.26	1.10 ± 0.68
	0.65 a_w	1.12 ± 0.15	1.47 ± 0.22	2.81 ± 0.77
Almond meal	0.25 a_w	0.17 ± 0.08	0.25 ± 0.06	0.21 ± 0.12
	0.45 a_w	0.37 ± 0.36	0.51 ± 0.30	0.52 ± 0.20
	0.65 a_w	0.16 ± 0.11	0.34 ± 0.14	0.68 ± 0.20
Almond butter	0.25 a_w	0.07 ± 0.37	0.07 ± 0.17	0.32 ± 0.19
	0.45 a_w	0.89 ± 0.84	1.01 ± 0.84	0.99 ± 0.58
	0.65 a_w	0.53 ± 0.17	0.86 ± 0.08	1.30 ± 0.22

Table I.2 *Salmonella* population (± standard deviation) reduction during the thermal come-up time for wheat products.

Products		*Salmonella* population (log CFU/g)		
		80°C	85°C	90°C
Wheat kernels	0.25 a_w	0.20 ± 0.35	0.21 ± 0.53	0.05 ± 0.54
	0.45 a_w	0.51 ± 0.61	0.68 ± 0.31	0.92 ± 0.60
	0.65 a_w	0.30 ± 0.12	0.37 ± 0.20	0.97 ± 0.19
Wheat meal	0.25 a_w	0.07 ± 0.19	0.24 ± 0.16	0.32 ± 0.22
	0.45 a_w	0.02 ± 0.35	0.55 ± 0.27	1.38 ± 0.39
	0.65 a_w	0.70 ± 0.76	1.13 ± 0.25	3.04 ± 1.01
Wheat flour	0.25 a_w	0.14 ± 0.14	0.19 ± 0.10	0.67 ± 0.14
	0.45 a_w	0.11 ± 0.08	0.68 ± 0.42	2.04 ± 0.23
	0.65 a_w	0.40 ± 0.12	1.25 ± 0.07	3.41 ± 0.42

Table I.3 *Salmonella* population (± standard deviation) reduction during the thermal come-up time for date products.

Products		*Salmonella* population (log CFU/g)		
		70°C	75°C	80°C
Date pieces	0.25 a_w	0.72 ± 0.12	0.11 ± 0.97	0.89 ± 0.67
	0.45 a_w	1.18 ± 0.47	0.92 ± 1.12	0.22 ± 0.45
	0.65 a_w	1.50 ± 1.18	1.59 ± 0.25	1.95 ± 0.62
Date paste	0.25 a_w	0.08 ± 0.07	0.04 ± 0.07	0.16 ± 0.04
	0.45 a_w	0.22 ± 0.14	0.13 ± 0.05	0.75 ± 0.34
	0.65 a_w	0.29 ± 0.09	0.29 ± 0.12	2.89 ± 0.56

Appendix J. Shape Factor in Weibull Model

This appendix shows the relationship between shape factor and temperature/a_w for all products.

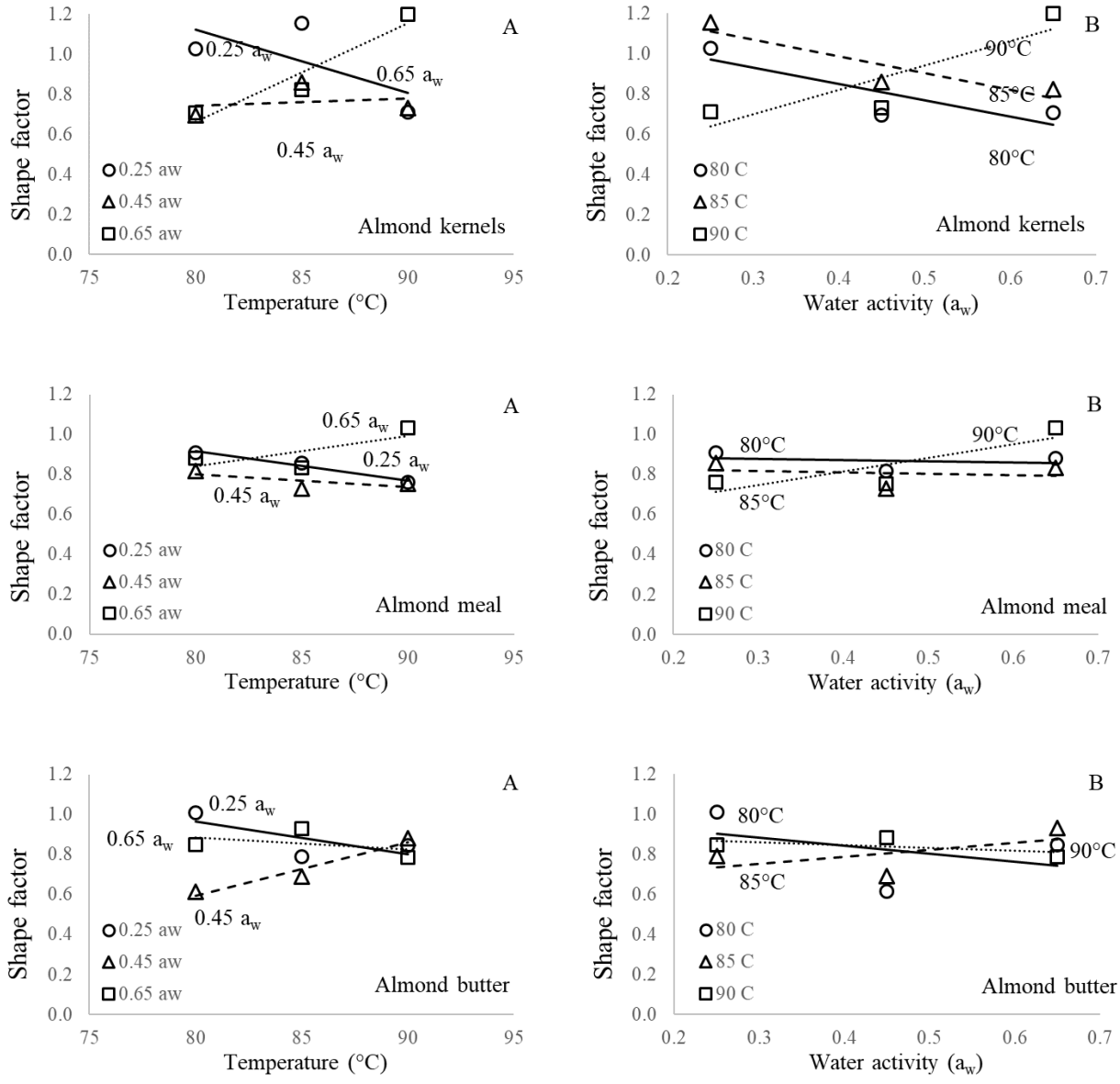

Figure J.1 Relationship of Weibull shape factor with (A) temperature (B) a_w for almond kernels, almond meal, and almond butter.

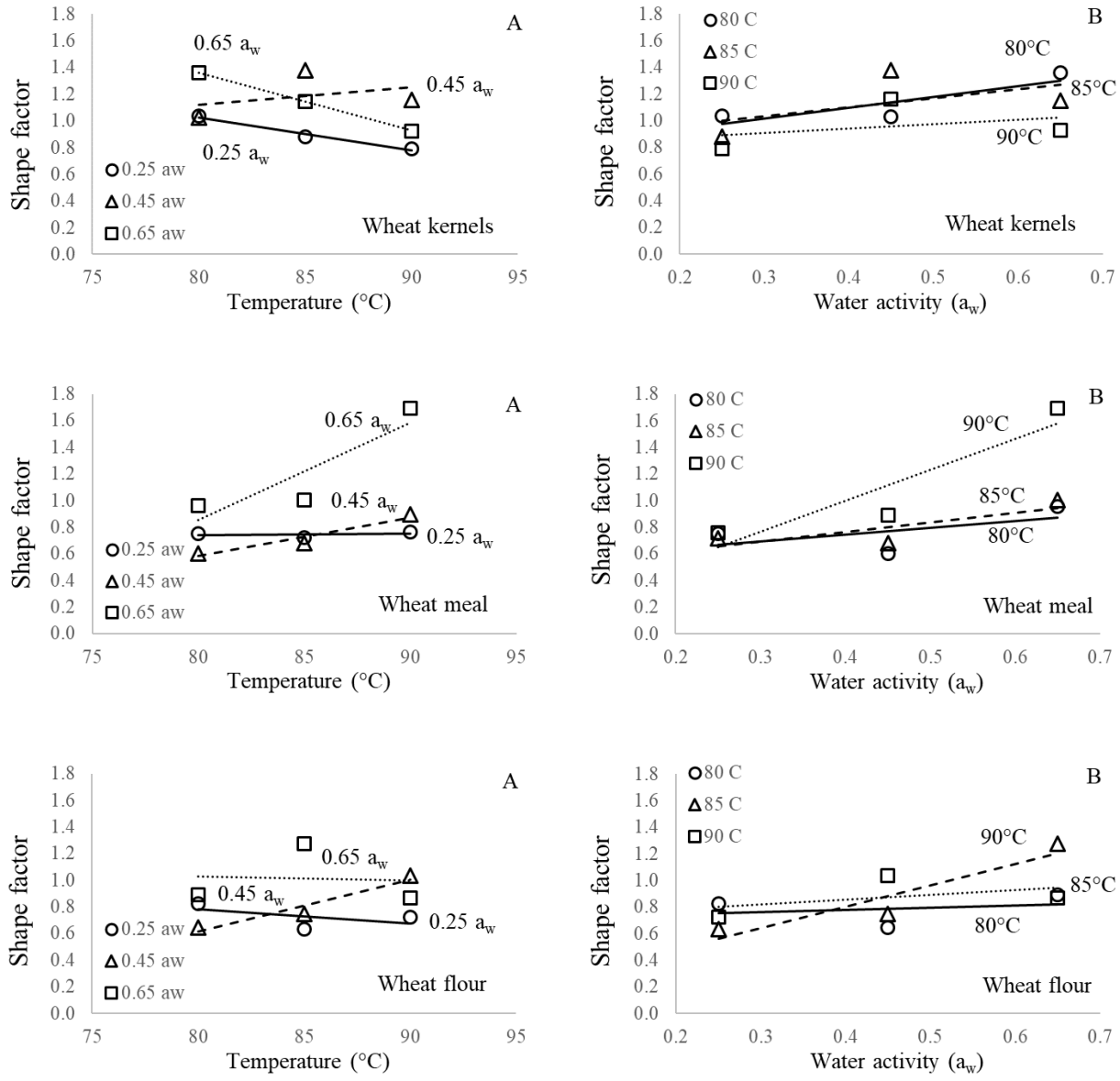

Figure J.2 Relationship of Weibull shape factor with (A) temperature (B) a_w for wheat kernels, wheat meal, and wheat flour.

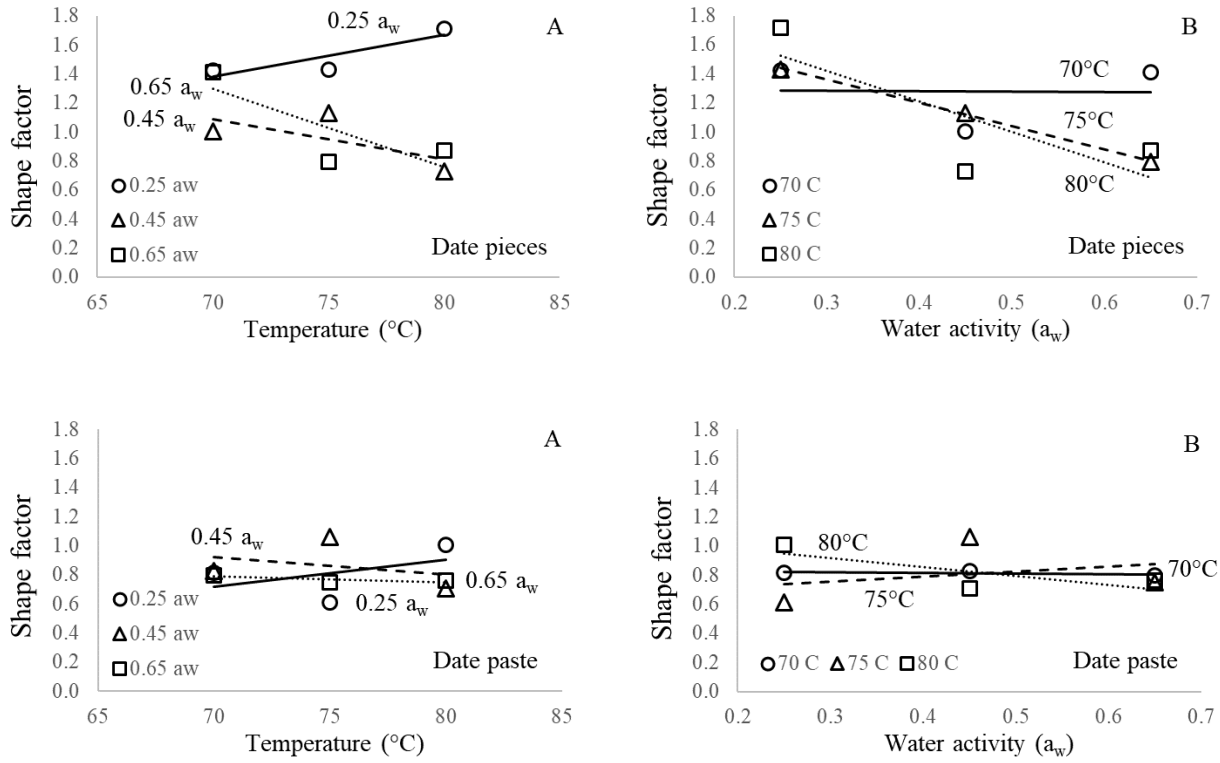

Figure J.3 Relationship of Weibull shape factor with (A) temperature (B) a_w for date pieces and date paste.

Appendix K. Scaled Sensitivity Coefficient for the log-linear/Bigelow-Type Model

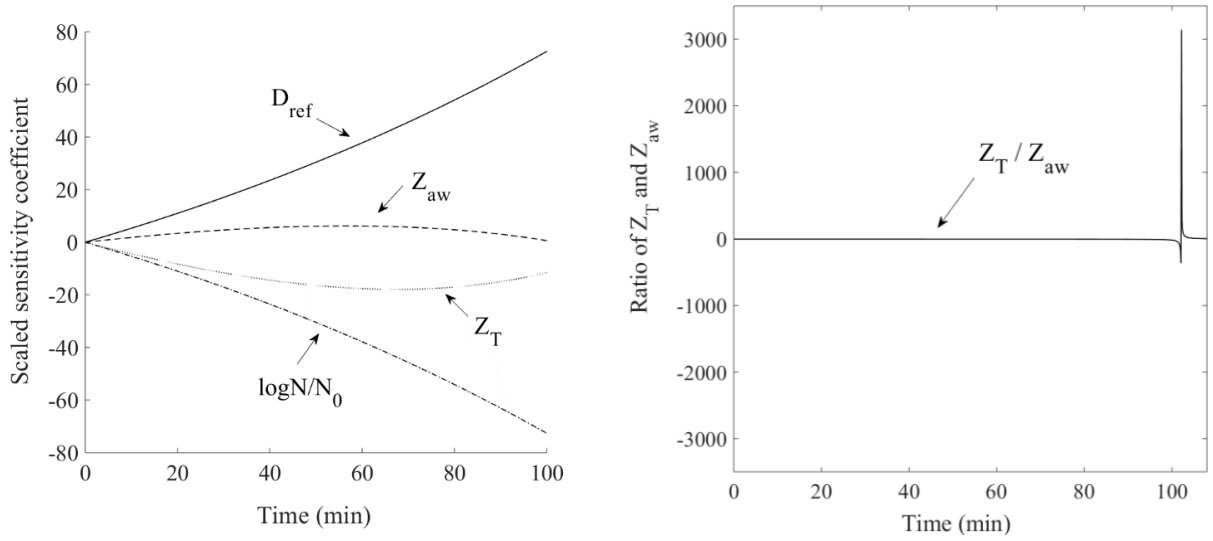

Figure K.1 SSC for the log-linear/Bigelow-type model of almond kernels.

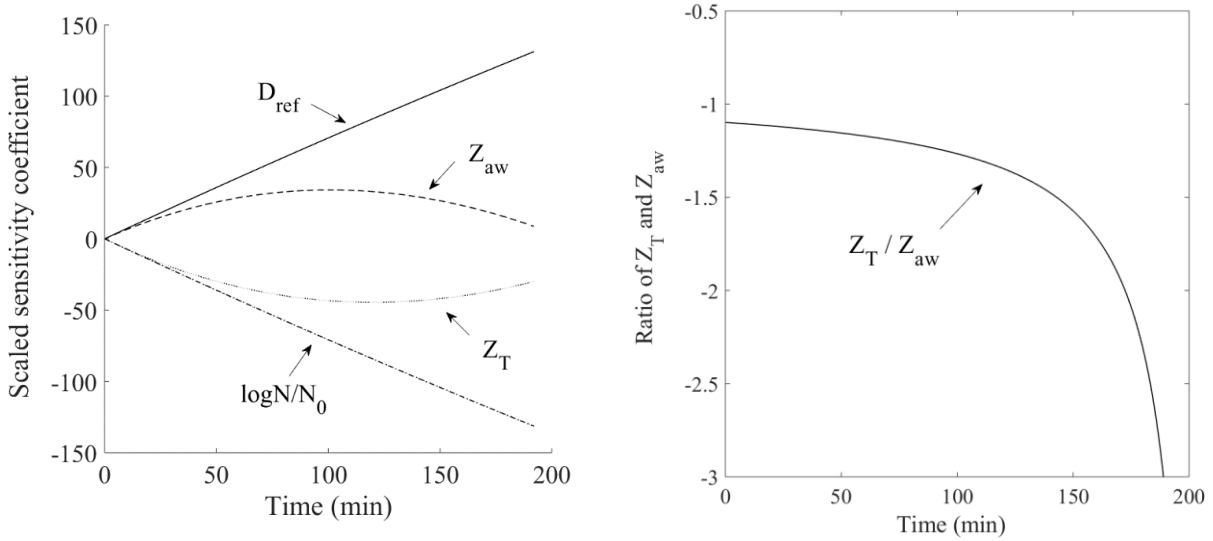

Figure K.2 SSC for the log-linear/Bigelow-type model of almond meal.

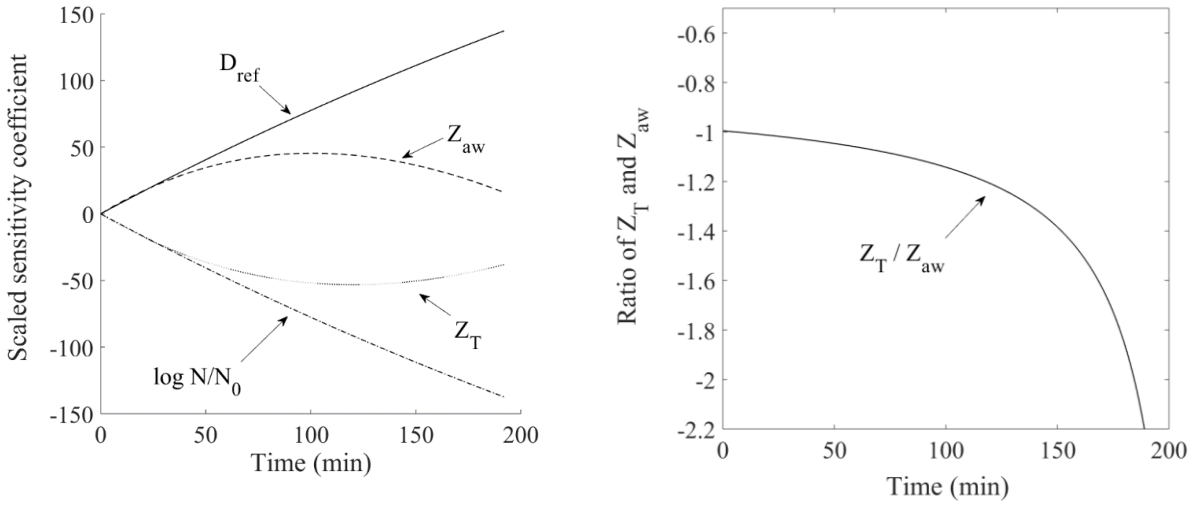

Figure K.3 SSC for the log-linear/Bigelow-type model of almond butter.

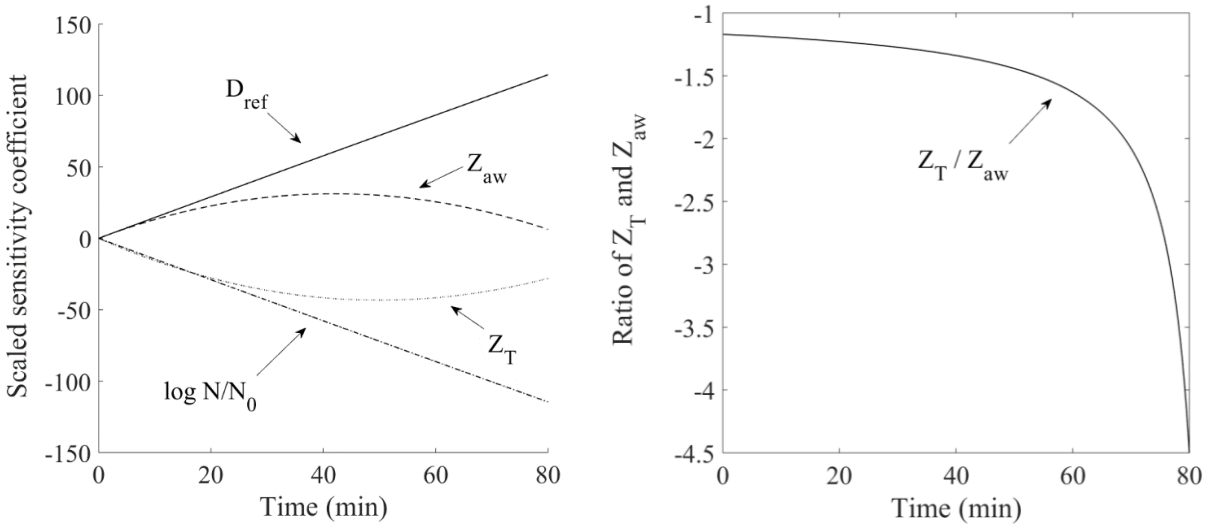

Figure K.4 SSC for the log-linear/Bigelow-type model of wheat kernels.

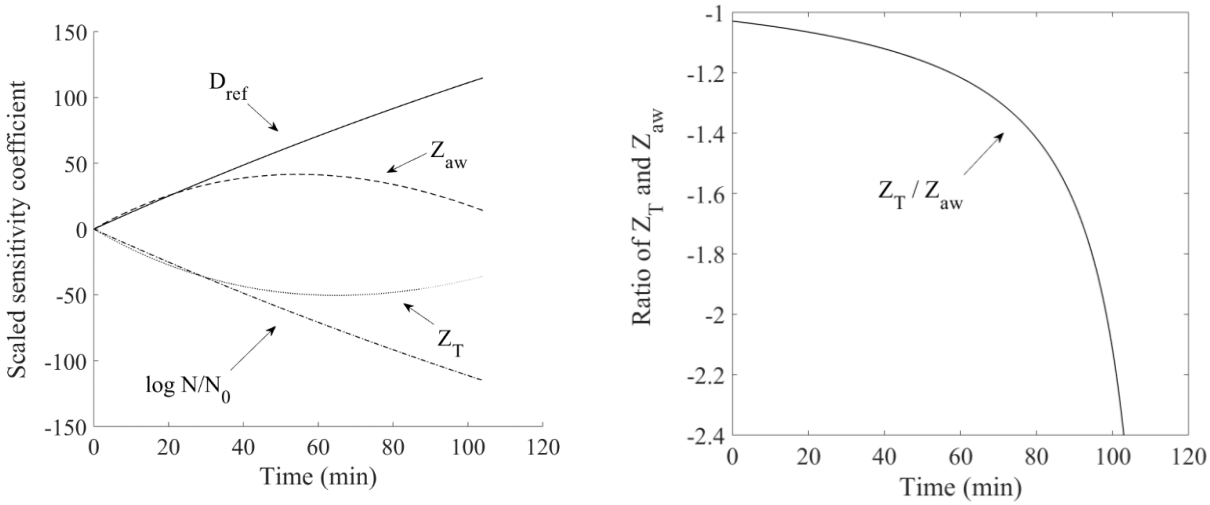

Figure K.5 SSC for the log-linear/Bigelow-type model of wheat meal.

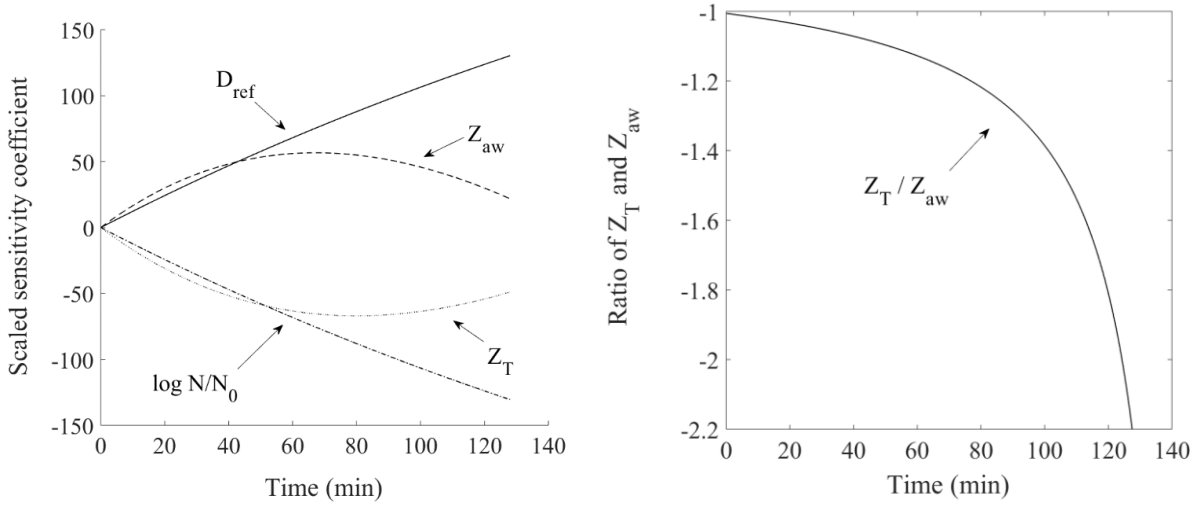

Figure K.6 SSC for the log-linear/Bigelow-type model of wheat flour.

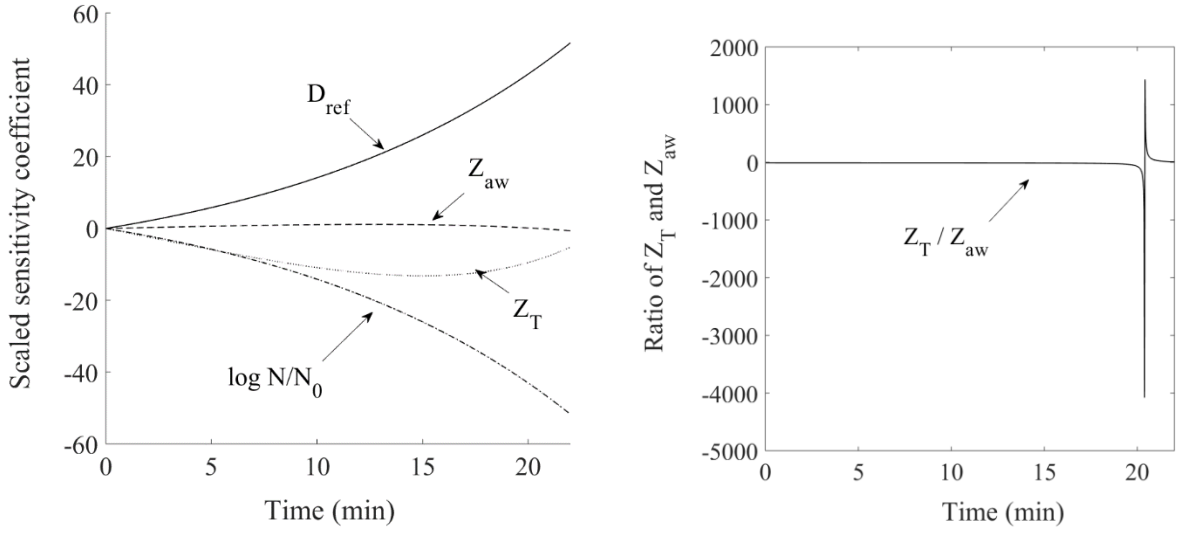

Figure K.7 SSC for the log-linear/Bigelow-type model of date pieces.

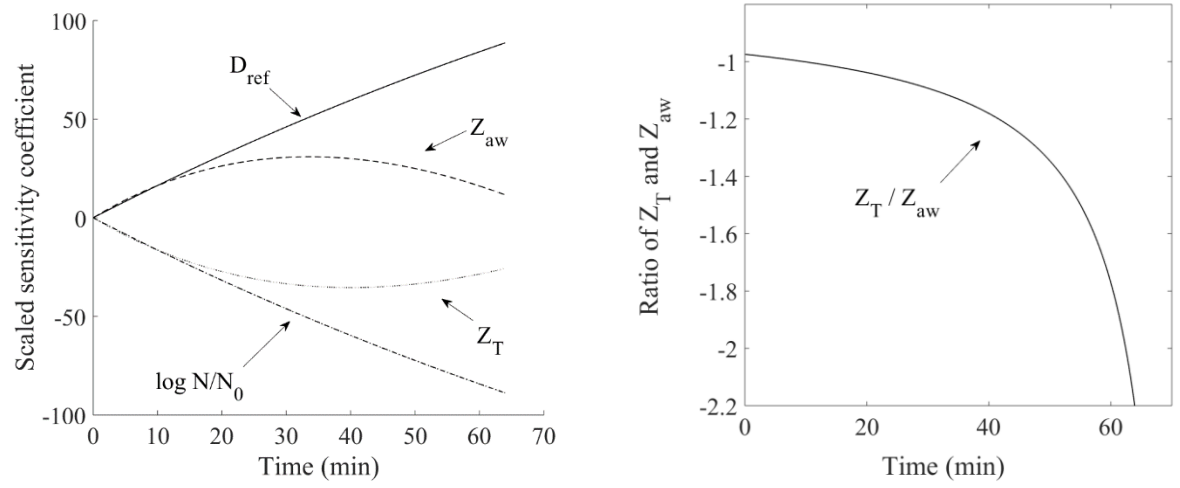

Figure K.8 SSC for the log-linear/Bigelow-type model of date paste.

Appendix L. Differential Scanning Calorimetry

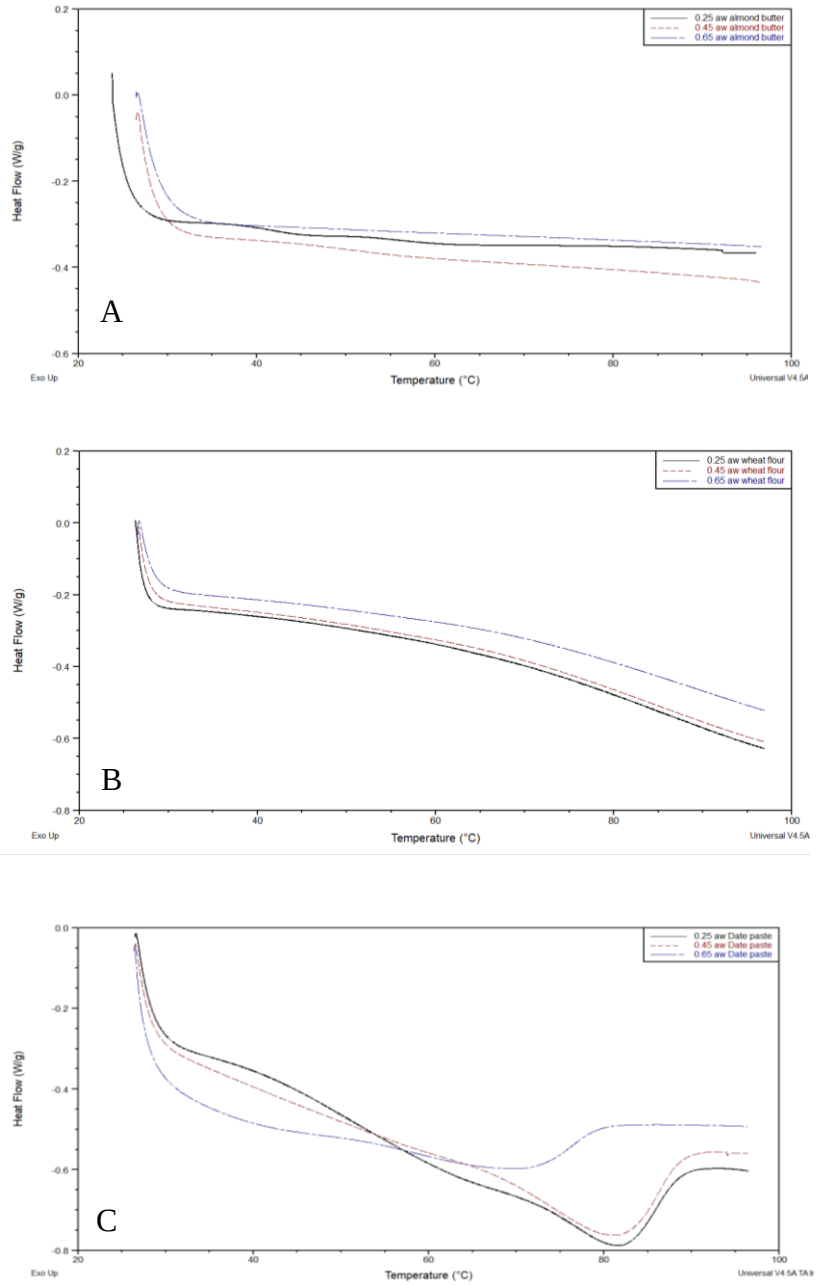

Figure L.1 DSC thermogram of (A) almond butter (0.25, 0.45, and 0.65 a_w), (B) wheat flour (0.25, 0.45, and 0.65 a_w), and date paste (0.25, 0.45, and 0.65 a_w).

Appendix M. Effects of the Fabrication Process on the Water Properties in Almond Products not Subjected to Complete Equilibration.

To quantify the change in a_w during the fabrication process, the almond kernels were equilibrated to 0.25, 0.45, and 0.65 a_w and then milled into almond meal and almond butter products as described in Chapter 5. In addition, natural almonds (almonds stored at room temperature) were also fabricated using the same method. The a_w and moisture content were measured using three replicates for all of the products.

The moisture content of the almonds in all of the products were stable ($P > 0.05$) after being milled into almond meal and almond butter (Table M.1), except for the 0.25 a_w sample ($P < 0.05$), which was due to the sample needing a longer amount of time to come to equilibration. The 0.25 a_w samples has the longest equilibration time because of the time required to decrease the water content (desorption) of the sample.

The a_w of the natural almonds (as received from supplier) was equivalent ($P > 0.05$) for all stages of the milling process; however, the a_w of the almonds that were equilibrated to the targets of 0.25, 0.45, and 0.65 a_w did change ($P < 0.05$) after milling into meal and butter. The changes in a_w after going through the milling process was likely due to non-uniform water distribution inside the large particles (i.e., incomplete equilibration), which caused the measured change in a_w of the almond meal and butter. These results show that the fabricated samples required a re-equilibration process in order to get back to the target a_w prior to the milling process.

Table M.1 The a_w and moisture content (± standard deviation) of almond kernels, almond meal, and almond butter fabricated from incompletely equilibrated almonds.

Initial a_w and product structure		a_w	Moisture content (%)
0.25 a_w	Almond kernels	0.244 ± 0.003^{A}	2.25 ± 0.07$^{A, B}$
	Almond meal	0.227 ± 0.013^{B}	2.05 ± 0.12^{B}
	Almond butter	0.350 ± 0.058^{B}	2.38 ± 0.16^{A}
Natural a_w	Almond kernels	0.440 ± 0.019^{A}	3.38 ± 0.23^{A}
	Almond meal	0.409 ± 0.018^{A}	3.16 ± 0.17^{A}
	Almond butter	0.420 ± 0.004^{A}	3.23 ± 0.13^{A}
0.45 a_w	Almond kernels	0.442 ± 0.006^{A}	3.95 ± 0.44^{A}
	Almond meal	0.448 ± 0.019^{A}	3.84 ± 0.63^{A}
	Almond butter	0.362 ± 0.032^{B}	3.69 ± 0.14^{A}
0.65 a_w	Almond kernels	0.665 ± 0.002^{A}	5.72 ± 0.33^{A}
	Almond meal	0.649 ± 0.012^{A}	5.19 ± 0.50^{A}
	Almond butter	0.575 ± 0.044^{B}	5.17 ± 0.59^{A}

Within a column and at the same a_w, values with a common superscript letter were not significantly different ($\alpha = 0.05$).

Appendix N. Effect of Almond Skin Integrity on *Salmonella* Thermal Resistance

This appendix was partially presented in a poster at the 2015 International Association for Food Protection (IAFP) Annual Meeting (Limcharoenchat et al., 2015).

To evaluate and quantify the effect of skin integrity on *Salmonella* thermal resistance, almonds were tested with their skin as either whole (fully intact), skin-damaged (partially intact), or blanched (absent). The skin-damaged almonds were produced using a vibratory tumbler (Model 67617, Central Machinery Inc., China) to shake the almond kernels (100 g) for 45 min. Silicon carbide sandpaper (Grit #36, Rust-Oleum, Illinois, USA) was glued inside the tumbler to partially remove the almond skin. The blanched almonds were produced by placing raw almonds (100 g) into hot water (100°C) for 1 min. The almond skins were then peeled off, excess water removed, and then the almonds were placed in a biosafety cabinet for 1 h (air speed ~0.33-0.38 m/s) to dry. Whole, skin-damaged, and blanched almonds all were surface-inoculated with *Salmonella* Enteritidis PT 30, equilibrated to ~0.40 a_w, and thermally treated at 80°C as described in Chapter 4.

Salmonella inactivation curves were calculated for each product (Figure N.1). The $D_{80°C}$ (± standard error) of the whole, skin-damaged, and blanched almonds were 20 ± 4.5 min, 19.2 ± 1.3 min, and 17.9 ± 3.9 min, respectively. The statistical analysis (ANOVA) results indicated that the *Salmonella* thermal resistance on whole, skin-damaged, and blanched almonds were equivalent ($P > 0.05$). These results indicate that skin integrity of an almond does not have an impact on *Salmonella* thermal resistance on almond surface. Similar product structure may have similar influence on the thermal resistance.

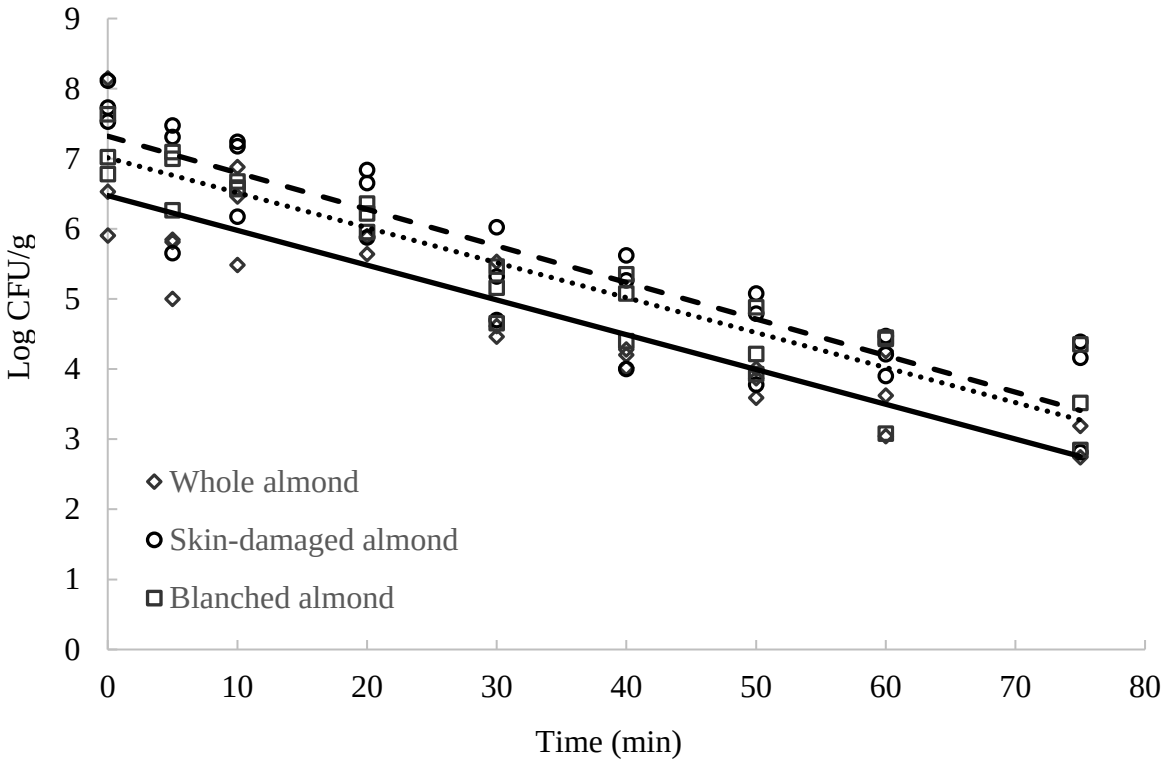

Figure N.1 Survival (log CFU/g) of *Salmonella* Enteritidis PT30 during isothermal heating (~80°C) of whole, skin-damaged, and blanched almonds (~0.40 a_w).

Appendix O. Effects of Equilibration Protocol, Water Properties, and Product Structure on *Salmonella* Thermal Resistance on/in Almond Kernels, Almond Meal, and Almond Butter

This appendix was presented in a poster at the 2016 IAFP Annual Meeting (Limcharoenchat et al., 2016).

To quantify the effects of water properties and product structure on *Salmonella* Enteritidis PT30 on/in almond kernels, almond meal, and almond butter, almond kernels were inoculated and partially or fully equilibrated to 0.25 a_w (Table O.1) before testing *Salmonella* thermal resistance at 80°C as described in Chapter 5.

Table O.1 Definition of partial and full equilibration of almond kernels, almond meal, and almond butter.

Sample	Partial equilibration	Full equilibration
Almond kernels	Surface a_w of the almond was measured (~0.25 a_w).	Surface a_w of almond was measured before splitting the almond in half. Split almond a_w (called internal a_w) was measured. Difference between surface and internal a_w was < 0.04.
Almond meal	Equilibrated almond kernels (100 g) were ground (45 s) into meal.	Almond meal was re-equilibrated in controlled-environment chambers (~2 days).
Almond butter	Equilibrated almond kernels (200 g) were milled (16 min) into butter. Dry ice added every 2 min to control product temperature (<40˚C)	Almond butter was re-equilibrated in controlled-environment chambers (~4-7 days).

The a_w and moisture content of the almond products before heating (Table O.2) indicate that the a_w of the samples for partial equilibration was significantly different ($P < 0.05$), but the moisture content was equivalent ($P > 0.05$). These results show that the desorption of the whole almonds introduced the variation of the a_w after fabrication.

Table O.2 The a_w and moisture content (± standard deviation) before heating of almond kernels, almond meal, and almond butter after partial and full equilibration.

Product		a_w	Moisture content (%)
Partial equilibration	Almond kernels	$0.245 \pm 0.011^{B, C}$	4.07 ± 0.47^{A}
	Almond meal	0.285 ± 0.005^{A}	$3.57 \pm 0.22^{A, B}$
	Almond butter	0.217 ± 0.047^{C}	$3.38 \pm 0.23^{A, B}$
Full equilibration	Almond kernels	$0.254 \pm 0.011^{A, B}$	$3.49 \pm 0.05^{A, B}$
	Almond meal	$0.251 \pm 0.010^{A, B}$	2.20 ± 0.58^{C}
	Almond butter	$0.251 \pm 0.008^{A, B, C}$	$2.86 \pm 0.05^{B, C}$

Within a column, values with a common superscript letter were not significantly different ($\alpha = 0.05$).

Salmonella thermal resistance (Figure O.1 and Table O.3) of almond meal for partial equilibration was lower ($P < 0.05$) than the thermal resistance at full equilibration because of the higher a_w. These results suggest that the re-equilibration process was necessary for controlling the effect of a_w on *Salmonella* thermal resistance.

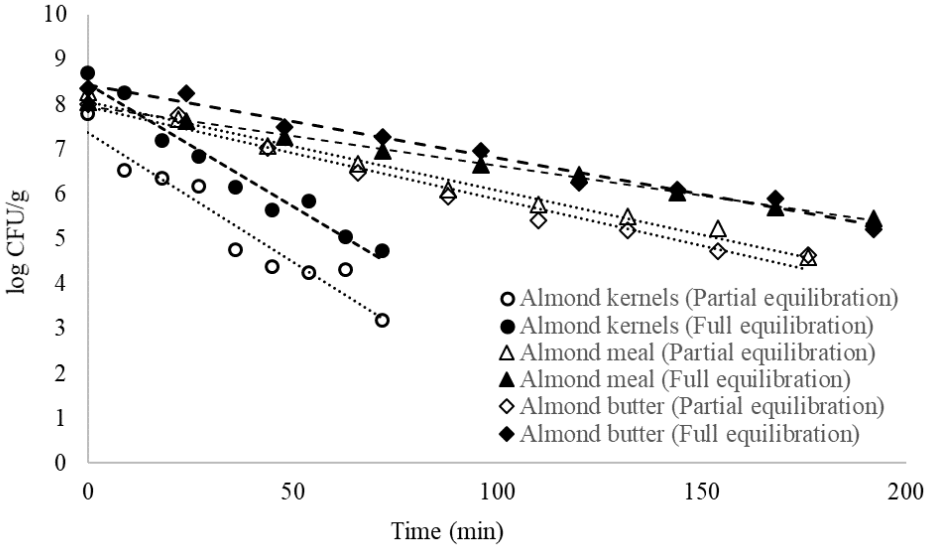

Figure O.1 Survival **(**log CFU/g) of *Salmonella* Enteritidis PT30 during isothermal heating (~80°C) of the almond kernels, meal, and butter after partial and full equilibration (~0.25 a_w).

Table O.3 $D_{80°C}$ values (± standard deviation) determined by linear regression of the *Salmonella* survivor curves (Figure O.1) of the almond kernels, almond meal, and almond butter after partial and full equilibration (~0.25 a_w).

Product		$D_{80°C}$ (min)
Partial equilibration	Almond kernels	18.0 ± 4.2^{C}
	Almond meal	51.9 ± 8.7^{B}
	Almond butter	48.6 ± 3.5^{B}
Full equilibration	Almond kernels	18.8 ± 2.6^{C}
	Almond meal	76.7 ± 13.2^{A}
	Almond butter	62.1 ± 6.9$^{A, B}$

Parameters with the same superscript letter were not significantly different (α = 0.05).

Appendix P. Effect of the Type of Inactivation Container Used on *Salmonella* Thermal Resistance

To quantify the effect that inactivation containers used in this study had on *Salmonella* Enteritidis PT30 (almond kernels, almond butter, wheat kernels, date pieces and date paste) samples were inoculated, equilibrated (~0.45 a_w), packed into test cells and plastic bags (Table P.1) before thermally treating the *Salmonella* at 80°C as described in Chapter 5.

Table P.1 Inactivation container loading for almond kernels, almond kernels, almond butter, wheat kernels, date pieces and date paste.

Sample	Test cell	Plastic bag
Almond kernels	A single almond was cut into small pieces and loaded for full coverage in the test cell.	A single almond was vacuum-packed in a plastic bag (See Chapter 5).
Almond butter	Almond butter was loaded into a test cell (See Chapter 5).	Almond butter was loaded into a plastic bag before sealing (20 x 20 x 1 mm).
Wheat kernels	Seven wheat kernels were loaded into a test cell.	Seven wheat kernels were vacuum-packed into a plastic bag (See Chapter 5).
Date pieces	One date piece was loaded into a test cell.	One date piece was vacuum-packed into a plastic bag (See Chapter 5).
Date paste	Date paste was loaded into a test cell (See Chapter 5).	Using a test cell, date paste was shaped and sized before vacuum-packing in plastic bag.

Salmonella inactivation curves (Figure P.1) of each product type were compared by using the analysis of covariance (ANCOVA) in MATLAB. Results suggested that *Salmonella* thermal resistance of almond butter, date pieces and date paste in both containers were not significantly different ($P > 0.05$); however, *Salmonella* thermal resistance of almond and wheat kernels in test cells were greater ($P < 0.05$) than in plastic bags. However, the whole almond kernels actually had to be broken into pieces to fit into the test cells, and that they were no longer truly whole almonds. These results suggested that the inactivation container did not have an impact on *Salmonella* thermal resistance when they have the same surface area of the samples in contact with the container.

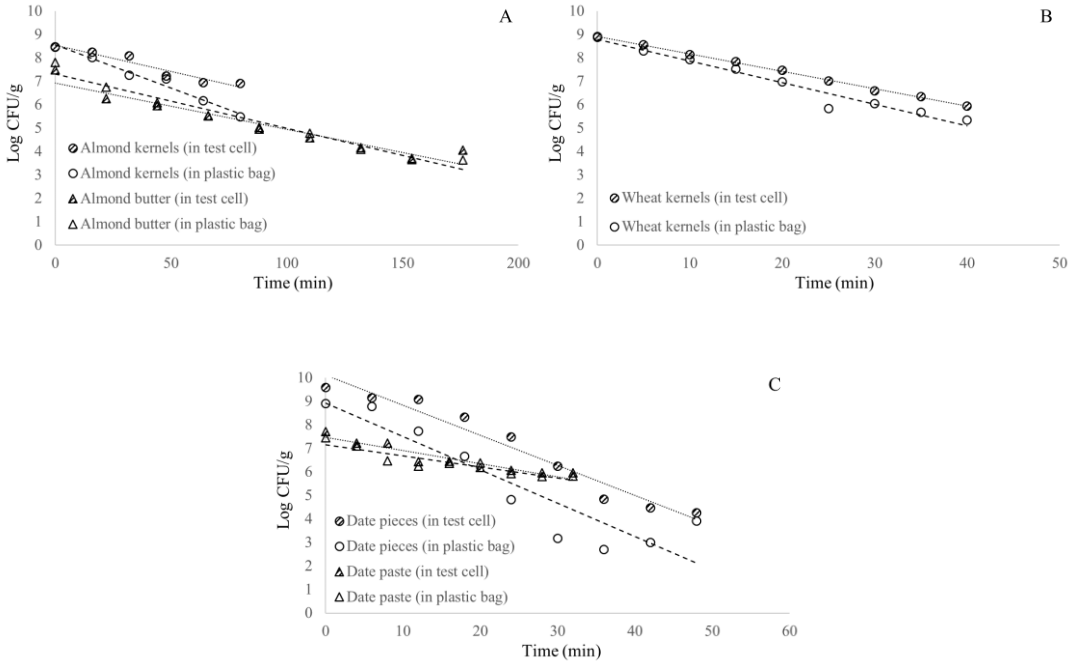

Figure P.1 Survival **(**log CFU/g) of *Salmonella* Enteritidis PT30 during isothermal heating (~80°C) of (A) almond products (~0.25 a_w), (B) wheat kernels (~0.45 a_w), and (C) date products (~0.45 a_w).

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