MODELING THE GROWTH OF *PSEUDOMONAS PUTIDA* AND *SALMONELLA ENTERICA* IN RAW GROUND POULTRY MEAT SUBJECTED TO A DYNAMIC TEMPERATURE DEVIATION

By

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(Under the Direction of Manpreet Singh)

ABSTRACT

This research was conducted to develop and validate growth models for *Pseudomonas putida* and *Salmonella enterica* in raw ground poultry meat. Separate experiments included similar experimental methods by inoculating bacteria into irradiated raw ground chicken meat; subjected to isothermal temperatures (*P. putida* at 5-35°C and *S. enterica* at 7-45°C) within the entire growth temperature range to simulate temperature fluctuation and possible deviations. Microorganisms were quantified and fitted with the Baranyi equation using USDA-ARS Integrated Pathogen Modeling Program to create a primary model and yield a low RMSE value for both microorganisms (0.065-0.519), and the maximum specific growth rate (μ_{max}) to create the secondary model. Secondary model used the Ratkowsky square root equation resulting in RMSE values for *P. putida* (0.05) and *S. enterica* (0.08), indicating high reliability. Tertiary models were validated with dynamic sinusoidal low and high temperature profiles: *P. putida* (5-20°C and 15-35°C) and *S. enterica* (7–25 °C and 20–45 °C). Validated tertiary models demonstrated a strong goodness-of-fit between observed data and predicted values.

INDEX WORDS: *Pseudomonas*, *Salmonella*, predictive modeling, spoilage, poultry

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DEDICATION

To my family and friends, whose endless love, encouragement, and belief in me has been my foundation throughout this journey.

To my mentors and educators, thank you for your guidance, inspiration, and for teaching me vital lessons I could not have learned on my own.

This work would not have been possible without your unwavering support.

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Chapter 1

INTRODUCTION

Poultry meat is the most consumed animal protein since 2015, and the National Chicken Council (NCC) reports 115.9 lbs. per capita consumption of poultry in 2023 (*National Chicken Councils*, 2021). The shelf life of meat products is strongly influenced by the packaging type, meat form, and storage temperature. The average shelf life of refrigerated fresh meat products is one to two days from the date of purchase; however, this can vary depending on company-specific interventions to extend shelf-life and the amount of time elapsed between the final packaging step of the processing chain and the arrival of product to retail stores. Previous research includes the investigation of spoilage bacteria growth in raw meat products for up to 25 days of storage when product is treated with various preservation methods (Bruckner et al., 2013; Chouliara et al., 2007; Mexis et al., 2012; Saucier et al., 2000). Spoilage of meat occurs when products are stored outside their recommended timeframe or temperature, resulting in undesirable sensory changes in the product, such as the development of foul odors, discoloration, and liquid accumulation. Ground meat products have a shorter cold storage shelf life of one to two days when compared to three to five days in whole beef cuts *(US Department of Health and Human Services*, 2019). This reduced shelf life can be attributed to the significantly increased surface area exposure to processing equipment, thus providing more opportunity for bacterial growth and accelerated oxidative processes. The increased incidence of ground poultry spoilage

compared to beef can be due to the higher water activity (FSIS, 2025). Because water is a component of protein and not a component of fat, poultry meat is more prone to spoilage because of its naturally higher lean percentage. A 2010 study estimated 22 billion lbs. of poultry meat produced, 21.8 % of which was lost to waste by several factors, including spoilage by *Pseudomonas* spp. during temperature abuse and contamination of the pathogenic bacteria, *S.* enterica (*Buzby et al., 2014)*. *Pseudomonas* spp. remain the leading bacterial organism contributing to the spoilage due to its ubiquitous and psychrotrophic properties that allow it to adapt and persist in raw poultry products.

Food waste contributes not only to substantial direct economic losses but also to significant indirect costs, including waste management, labor, and greenhouse gas emissions. In response to the billions of dollars in economic losses and public health impact from foodborne pathogens in the past, several studies have been conducted to develop mathematical models to predict the growth of specific pathogens, including *S. enterica*, during improper temperature storage conditions (Juneja et al., 2007). These models are being developed to accurately predict the risk of pathogenic bacterial growth to reduce the potential for foodborne illness. This method of approximate enumeration can be used as an instant preliminary assessment before investing more time-consuming and extensive testing when the growth of pathogenic and spoilage microorganisms due to temperature abuse is suspected. The purpose of this study was to develop predictive models to determine the growth of *Pseudomonas putida* and *Salmonella enterica* and establish a tool for users to employ during risk analysis of pathogen contamination and spoilage following temperature deviation. While establishing a predictive model for *Pseudomonas* spoilage in ground poultry meats is critical to prevent significant economic losses, modeling and validating the growth of *Salmonella* is important from a public health perspective and adds to the

value of this research by providing a more accurate and widespread representation of temperature deviation in ground poultry products. The following were the main objectives of the research:

1. To estimate the behavior and growth curve of *Pseudomonas putida* and *Salmonella enterica* based on previous modeling study data and growth characteristics observed from the literature review.
2. To establish a minimum and maximum growth temperature for each organism during isothermal temperature monitoring.
3. To develop a mathematical predictive model for the growth of *Pseudomonas putida* under a full growth temperature profile (5°C-35°C)
4. To develop a mathematical predictive model for the growth of *Salmonella enterica* under a full growth temperature profile (7°C-45°C)
5. To validate each of these models while being subjected to two dynamic temperature profiles: a higher and a lower profile.

Outcomes from this study will establish a predictive modeling tool to aid in the development of an effective HACCP (Hazard Analysis and Critical Control Point) plan by setting critical limits and corrective actions based on realistic operational conditions. With accurate regulatory thresholds in place, these models will serve as an enhanced risk management application when presented with storage temperature deviations and offer earlier detection of spoiled product. This early detection can directly mitigate the costs associated with product loss due to microbial spoilage. These models also have the potential to provide a wider framework for shelf-life determination, thus resulting in a reduction of poultry meat spoilage and an increase in food sustainability. Ultimately, the predictive modeling tools established from this study will

play an essential role in providing oversight in both health-related and spoilage risks in poultry products by minimizing *Salmonella* illnesses and economic losses.

Chapter 2

LITERATURE REVIEW

2.1 Introduction to *Pseudomonas* spp.

Pseudomonas spp. are Gram-negative, facultative anaerobes that do not typically pose a serious health threat to the public following consumption in foodborne strains (Mellor et al., 2011). *Pseudomonas* is considered a naturally occurring organism because it is commonly present in soil, water, and areas of higher humidity. However, it is also frequently associated with the spoilage of animal-based protein products such as dairy, eggs, and meat due to its thermo-tolerant properties during pasteurization, psychrotrophic characteristics, lipolytic and proteolytic enzymes (Neumeyer et al., 1997). *Pseudomonas* spp. are able to tolerate and persist in stressful environments where the growth of other microorganisms may be inhibited (Wickramasinghe et al., 2019). The enzymatic properties of lipase and lecithinase from this organism allow for the degradation of nutrients within food systems, which generate useable metabolites for *Pseudomonas* to thrive. The combination of enzymatic activity along with other mechanisms, such as biosurfactant production, leads to the spoilage of raw poultry products (Mellor et al., 2011).

Along with the preexisting enzymes that are associated with the growth of *Pseudomonas*, this organism can produce a byproduct categorized as biosurfactants, a compound that enhances

the persistence of cells by converting surrounding nutrients into functional substrates that support cell survival (Mellor et al., 2011). Other contributing physical factors of the meat leading to a higher risk for skin-on products are the harboring of bacteria in bird feather follicles and the interior of bones (Rouger et al., 2017). Because of the capability of *Pseudomonas* to degrade complex nutrient compounds into simpler structures, billions of dollars and pounds of poultry meat are lost because of waste due to adverse sensory characteristics, including foul odor and slime accumulation.

Pseudomonas putida is commonly associated with raw poultry meat spoilage during chilled aerobic conditions. While it may not be the only species of *Pseudomonas* present, it is likely the most representative of the bacterial species responsible for spoilage in meat (Heir et al., 2021; Hinton et al., 2004; Koutsoumanis et al., 2006). Spoilage by *pseudomonads* is evaluated by the degree of color change, odor production, and slime accumulation (Koutsoumanis et al., 2006). These characteristics remain the most predominant physical indicators of poultry spoilage and are the reason for poultry meat loss due to adverse sensory effects and lower consumer acceptability.

Research has demonstrated that the growth of *Pseudomonas* can range from temperatures of 4-42°C (LaBauve & Wargo, 2012). While this temperature growth range is entirely dependent on the specific intrinsic (pH, fat composition, $a_{w,}$ and initial bacterial population), extrinsic (temperature and oxygen availability), and processing conditions associated during growth, it raises a large concern for the poultry processing industry from a financial aspect due to the increased likelihood of spoiled product. Spoilage of meat products is most attributed to abusive storage temperature conditions that are >4°C. Although it is not common for raw poultry meat to

be transported at temperatures greater than 10°C (Gill et al., 2002), even recurring minimal temperature deviations above 4°C can promote the growth of *Pseudomonas* spp.

2.2 Persistence of *Pseudomonas* spp.

The spoilage of most aerobically stored chilled food products can be attributed to the growth of *Pseudomonas* spp. This species known to tolerate further processing steps such as pasteurization and antimicrobials (Quintieri et al., 2021; Thomassen et al., 2023). Limited research has been conducted on *Pseudomonas putida* resistance to food processing; however, studies of other species of *Pseudomonas* have reported the organism to develop resistance to peracetic acid, a commonly used antimicrobial in several industries, including the poultry and meat industry (Akinbobola et al., 2017). A study conducted by Akinbobola et al. (2017) evaluated the resistance of *P. aeruginosa*, a common nosocomial strain of *Pseudomonas* that is a contaminant in surgical equipment. The findings concluded that this organism can tolerate concentrations of PAA at up to 2500 ppm, predominantly due to its biofilm virulence mechanism (Akinbobola et al., 2017).

Pseudomonads synthesize lipolytic and proteolytic enzymes that facilitate the degradation of surrounding nutrient sources to contribute to the development of the extracellular matrix or biofilm. The enzymatic properties of lipase, a lipolytic enzyme, allow the breakdown of triacylglycerol (TAGs) into free fatty acids, which results in the dehydration of meat fats (Chandra et al., 2020). Lecithinase is a proteolytic enzyme that is responsible for the breakdown of animal protein into consumable nutrient sources for *Pseudomonas* (Schmidt et al., 1969). In medical device equipment, nutrient sources often originate from the accumulation of organic soil

compounds in textured or irregular surfaces (Akinbobola et al., 2017). This risk is also seen in the food industry, with the residual debris on food contact surfaces providing ample nutrient supplies. As the biofilms age, the structure is reinforced and becomes more robust. During disinfection, aged biofilms can interact with disinfectant agents through their extracellular matrix, which reacts with the agent before it can penetrate the bacterial cell surface. This interaction can hinder the disinfectant's ability to eliminate the microorganism, thus leading to the cell's survival and persistence (Akinbobola et al., 2017).

Another significant concern regarding antimicrobial resistance is the emergence of biocide resistance resulting from phenotypic adaptations (Vikram et al., 2015). Biocides are another category of disinfectant used by several industries to control the growth of biofilm activity and include chlorine, quaternary ammonium compounds, and aldehydes, all disinfectant compounds frequently used in the food industry. The resistance of *P. fluorescens* biofilms to biocides is a rising phenomenon that was investigated by Vikram et al. (2015), focusing on the role of efflux pumps and phosphonate degradation, and various metabolic activities. The findings concluded that the resistance of *P. fluorescens* to glutaraldehyde, another type of biocide disinfectant, increased with biofilm maturity. As a biofilm ages, it is believed that the level of exopolysaccharides and proteins increases, which limits the penetration of the disinfectant. This study explains that when the bacteria are exposed to the disinfectant, metabolic processes are triggered that further contribute to the growth of the biofilm, thus reinforcing its persistence.

Multiple studies investigating the role of biofilms in the resistance to disinfectants suggest that the resilient properties of *Pseudomonas* spp. continue to remain a significant concern across multiple industries such as health care and food processing. Whether it is medical

device equipment or food spoilage, this ubiquitous bacterium continues to present challenges, costing billions of dollars.

2.3 *Pseudomonas* spp. Biosurfactant Production

Mellor et al. (2011) define these biosurfactants as biologically produced amphipathic compounds with affinity for interfaces that reduce surface and interfacial tension. The properties associated with biosurfactants include emulsification, penetration, and microbial growth enhancement (Thavasi et al., 2011). Although there are more negative attributes associated with biosurfactant production in most applications, several studies have been conducted to investigate the role of biosurfactants in the degradation of crude oil pollution in contaminated environments, including soil and the ocean (Thavasi et al., 2011; Yagoo & Vilvest, 2023). A study conducted by Yagloo & Vilvest (2023) investigated the prevalence of *Pseudomonas aeruginosa* in soil samples. It provided findings of antimicrobial activity against various microbial species when inoculated with *P. aeruginosa* cultures with pre-existing biosurfactant compounds during an 'Antagonist test'. The *P. aeruginosa* strains were effective against *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*, all species commonly found in soil (Yagoo & Vilvest, 2023). In the poultry processing industry, biosurfactant compounds play a strong role in the emulsification of rendered chicken fats and can speed up the spoilage rate of chicken (Mellor et al., 2011). When biosurfactants are present, the increased spoilage rate could be attributed to the readily available skin-associated fat. Skin-on poultry products can harbor *pseudomonads* and other spoilage organisms in the feather follicles of the skin, which can make processing measures less effective (Rouger et al., 2017). This is supported by the slightly longer shelf life during cold

storage in skinless poultry cuts because of the lower lipid and hydrocarbon availability to be broken down.

2.4 *Pseudomonas* spp. Role in Spoilage of Ground Poultry Products

While *Pseudomonas* spp. and other spoilage organisms are not regulated in the food industry, they can still be identified as economic adulterants or indicators of poor sanitation. There are regulations in place from The United States Department of Agriculture Food Safety Inspection Service (USDA-FSIS) that require written sanitation and hygiene protocols such as Good Manufacturing Practices (GMPs) and Sanitation Standard Operating Procedures (SSOPs) that must meet the requirements of the Code of Federal Regulations §416.12, and perform necessary verification tasks described in FSIS directives (5000.1 and 5000.4). These required processes can aid in limiting the microbial contamination in foods. *Pseudomonas* spp. and other non-hazardous spoilage organisms are typically monitored through aerobic plate counts (APC) or psychrotrophic plate count in refrigerated products. This is because the growth of *Pseudomonas* or psychrotrophic bacteria could potentially indicate the growth of *Listeria monocytogenes*, a pathogen commonly found in chilled Ready To Eat (RTE) products (Thomassen et al., 2023).

Because of its combined virulence mechanisms to resist extensive processing conditions and antimicrobial interventions (Thomassen et al., 2023), it is the major contributor to the development of undesirable sensory characteristics. There are two types of poultry meat that are predicted to have the highest spoilage rate: skin-on poultry products and comminuted or ground poultry meat (Hinton et al., 2004). Because skin-on products can physically harbor more bacterial populations during the poultry harvesting process, thus resulting in a high microbial

load. Ground poultry and poultry meat products are more susceptible to spoilage due to the increased subjection to processing equipment and surface area contact compared to whole-meat cuts (Hinton et al., 2004). Rouger et al. (2017) mentions that a substantial amount of contamination of spoilage bacteria can be sourced from processing equipment surfaces located in a poultry processing facility, such as the rubber picking fingers used for feather removal (Figure 2.1) and conveyor belts (Arnold & Yates, 2009; Rouger et al., 2017). Both equipment parts include pronounced crevices and introduce favorable conditions for bacterial harborage and biofilm establishment (Arnold & Yates, 2009). Other areas of contamination identified during poultry operations include airborne bacteria, water from scalding and frequent rinsing and chilling steps, cross-contamination during evisceration, deboning, cutting, mincing, and further processing, including the addition of ingredients such as marinades (Rouger et al., 2017).

Because of the seemingly endless points of introduction for spoilage bacteria contamination during poultry processing, the likelihood of spoilage bacteria presence is nearly inevitable and raises significant concern from both a financial and sustainability perspective.

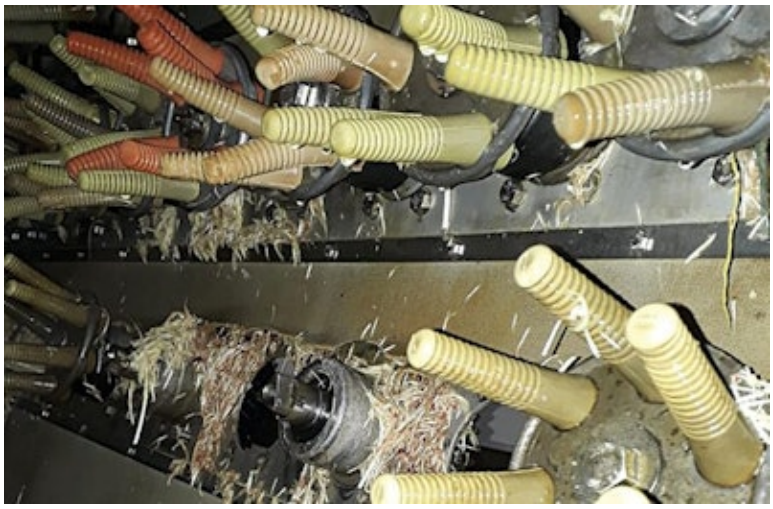

Figure 2.6: A picture by Eduardo Cervantes Lopez showing plucking fingers inside equipment (WATT Poultry, 2019)

2.5 Financial Impact of Food Spoilage in Poultry Products by *Pseudomonas* spp.

The USDA defines food loss as the edible amount of food, post-harvest, that is available for food consumption but is not consumed and disposed (Buzby et al., 2014). In 2010, the USDA's Economic Research Service estimated that approximately 30-40 percent of the United States food supply became food waste, weighing approximately 133 billion pounds and costing $161 billion (Buzby et al., 2014). Food waste can result from many stages of the production and supply chain; however, spoilage remains one of the top reasons. Spoilage of food can be attributed to many factors with microbial contamination remaining the most prominent. Microbial food spoilage is caused by bacteria, yeast and mold growth that facilitates metabolic process to breakdown the organic compounds causing off-odors, flavors, textures, and colors. While these unwanted sensory characteristics may not always cause a health concern, they are undesirable to consumers and result in the disposal of food products.

In 2010, the Economic Research Service (ERS) estimated that out of the 22 billion pounds of poultry meat produced, 21.8% was lost to waste, including microbial spoilage and contamination (Buzby et al., 2014). When food product is wasted, it is not only the physical nutritional components that are lost, but also the land area, water, labor, and energy that were put into the process of making that food available for consumption. The ERS refers to these indirect costs as "negative externalities" and defines them as costs accumulated from the spillover of another action that can adversely affect the environment and society (Buzby et al., 2014). Some of these externalities caused by food waste include increased pressure on the availability of natural resources, elevated greenhouse gas emissions from livestock operations and waste management processes, and contamination of the water supply. In 2015, the USDA partnered with the U.S. Environmental Protection Agency (EPA) to set a goal of cutting the nation's food

waste by 50 percent by the year 2030 (EPA, 2025). With five years left to meet this goal, food manufacturers are still struggling to implement changes in their operations to reduce the amount of food waste from both the producer and consumer side of the food industry.

2.6 Introduction to *Salmonella enterica*

Salmonella enterica is a Gram-negative facultative anaerobe that is a member of the *Enterobacteriaceae* family, and is currently the most common bacteria associated with foodborne illness (CDC, 2022; FDA, 2024). The United States Centers for Disease Control and Prevention (CDC) estimates that 1.35 million infections occur in the United States every year due to *Salmonella*, with approximately 23% of those illnesses linked to the consumption of poultry meat (CDC, 2024; Lamichhane et al., 2024). Salmonellosis is a bacterial disease that can result from consuming contaminated food products that may lead to severe illness and, in some cases, death. The most common serotypes of *Salmonella enterica* detected in poultry include *S.* Enteritidis, *S.* Heidelberg, *S.* Kentucky, *S.* Newport, and *S.* Typhimurium (CDC, 2022; Foley et al., 2011; Williams et al., 2025). There have been shifts in the most predominant serovar over the last several decades (Foley et al., 2011), and they interestingly show no consistency across time or geographical region (Williams et al., 2025). Research is being done to reduce the incidence of *Salmonella* in poultry meat, but this bacterium remains off the zero-tolerance microbial limit list in raw poultry processing because of its nearly guaranteed presence in testing. While the health concerns associated with *Salmonella* contamination remain of the highest priority, the economic impact is also of high concern. A recent study estimates that the occurrence of *Salmonella* in poultry meat leads to an estimated $2.8 billion in health issues, product waste, decline of life expectancy, etc. (Scharff, 2020). Because of the several negative attributes of *Salmonella*

becoming more apparent, new *Salmonella* performance standards in raw meat were proposed by FSIS as an effort to reduce the occurrence of *Salmonella* infections by 25% by the year 2030 (FSIS, 2025.) While this proposed framework may have been withdrawn because of several significant issues raised by representatives from various entities effected (FSIS, 2025), the concern for illnesses from *Salmonella* remains.

2.7 *Salmonella* Epidemiology

Salmonella enterica, or non-typhoidal *Salmonella,* is the current leading cause of bacterial illness and hospitalization following the consumption of contaminated food (Williams et al., 2025). The most common serotypes of *Salmonella enterica* infections from foodborne illness include Enteritidis, Typhimurium, and Newport (CDC, 2022; FDA, 2024; Foley et al., 2011; Williams et al., 2025). The CDC estimates that *Salmonella* causes approximately 1.35 million infections in the United States every year as of 2024. With chicken products remaining the second most prevalent source for *Salmonella* infection, the USDA estimates that one in every 25 packages of chicken meat in retail stores contains *Salmonella* (CDC, 2024). The CDC Active Surveillance Network reports that in 2022, approximately 15 in every 100,000 individuals were infected with *Salmonella* following the consumption of contaminated food (CDC, 2022). Of those illnesses, 27% of infections caused hospitalization, and less than 1% resulted in death(CDC, 2022). Over the last 10 years in the United States, *Salmonella* infections have peaked during summer months, and have had the highest incidence reports in the state of Georgia at 23% (CDC, 2022). Non-typhoidal Salmonella (NTS) is known to easily adapt to its host environment in order to quickly spread through the consumption of contaminated food and water because of the shorter incubation and shedding time (Lamichhane et al., 2024). This adaptation

in a host can be influenced by factors including initial infection dosage, susceptibility of the host, age of the host, host species, and *Salmonella* species (Lamichhane et al., 2024). For example, in most NTS infections, the pathogenesis includes a stronger ability to survive and withstand innate immune responses such as physical barriers (skin, mucosa membranes), physiological factors (body temperature, low pH), leukocytes (phagocytes, natural killer cells), and various resident enzymes and antimicrobial peptides (Haraga et al., 2008; Lamichhane et al., 2024).

2.8 *Salmonella* Infection

Salmonella bacteria can be classified into two categories*: S. enterica* and *S. bongori*, with *S. enterica* being one of the six subspecies that can be divided into over 2500 serovars (Achtman et al., 2012; Fookes et al., 2011). *S. bongori* is a species most associated with cold-blooded animals, and *S. enterica* is associated with disease in warm-blooded animals and humans (Fookes et al., 2011). There are two major categories for the *Salmonella enterica* subspecies: Typhoidal *Salmonella (TS)* and Non-Typhoidal *Salmonella (NTS)*. Non-typhoidal *Salmonella* includes the species *Salmonella Typhi* and *Salmonella Paratyphi* and results in the illness known as “Typhoid Fever”. This is a prolonged infection (1-3 weeks to show symptoms) that can lead to sepsis or a perforated intestine without treatment *(*Mayo Clinic, 2023). Salmonellosis is caused by non-typhoidal *Salmonella* bacteria, including the numerous serovars that can be categorized as Gastroenteritis or Extra-intestinal (Achtman et al., 2012). Illness is commonly associated with the serovars *S.* Typhimurium and *S.* Enteritidis.

Salmonellosis occurs following the ingestion of the bacteria and can occur through consuming contaminated food products that include, but are not limited to, meat, poultry, eggs,

milk, seafood, and fresh produce (CDC, 2024; Lamichhane et al., 2024). Consumption of contaminated food products accounts for approximately 95% of Salmonellosis, which is characterized by symptoms that include diarrhea, fever, chills, and abdominal pain (Foley and Lynn 2008).

These symptoms usually occur after an incubation period of six hours to six days and can persist for four to seven days (CDC, 2024; Lamichhane et al., 2024).

The infectious dose for *Salmonella* can range from 15-20 cells, but is normally at least 10,000 cells (Akil & Ahmad, 2019). While it is not as common, these symptoms can develop into septicemia (blood infections), arthritis, cholecystitis, endocarditis, etc. Death is unlikely, but it

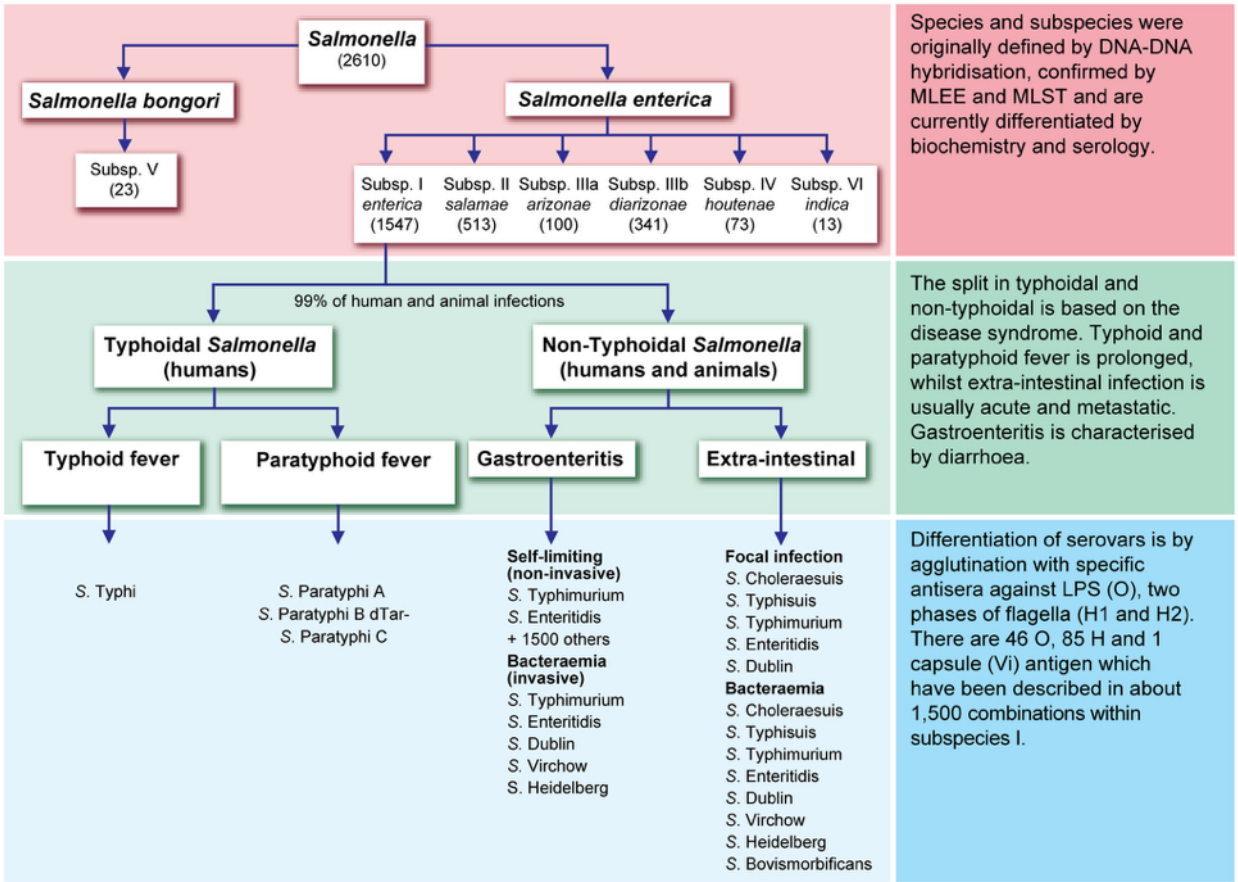

Figure 2.7: General overview of the current classification of *Salmonella enterica* (Achtman et al., 2012)

has been estimated that 400 fatalities occur per year in high-risk individuals such as the elderly, infants, and the immunocompromised (*Health & Human Services*, n.d.).

2.9 *Salmonella* Virulence Factors

2.9.1 Attachment and Internalization

The human digestive system, specifically the stomach, functions with fundamental defense mechanisms within the mucosal barrier that regularly provide protection from unwanted bacterial invasion. These defenses include low pH, the presence of specialized organelles (lysosomes), enzymes (lactoferrin, phospholipase, thiocyanate), and antimicrobial peptides (AMPS) that aid in the digestion of essential components of the pathogen, or sequester vital nutrients needed, such as iron (Haraga et al., 2008; Lamichhane et al., 2024). However, *Salmonella enterica* continues to withstand these defenses and cause infection through its adapted virulence mechanism. *Salmonella enterica* serovar Typhimurium employs the bacterial Type 3 Secretion System, which promotes adherence to the host cell and introduces effector molecules that inhibit signaling pathways for actin polymerization (Haraga et al., 2008). In doing so, the Salmonellae cause "ruffling" or the engulfment of the pathogen and internalization into the host (Figure 1; Haraga et al., 2008). *S. enterica* is most associated with the type 1 and curli fimbriae during bacterial adhesion; however, studies show that *S. enterica* can produce at least nine types of fimbriae (Althouse et al., 2003). Fimbriae are specific organelles resembling hairlike structures that line the outer membrane of the bacterial cell and allow for attachment to a host, and eventually biofilm formation (Rehman et al., 2019). Fimbriae are classified into three different categories based on their protein matrix pathway: Type 4, Chaperone-Usher (CU), and

Nucleated Precipitation (Rehman et al., 2019). Several previous studies suggest that T1F expression in *Salmonella enterica* serotype Typhimurium undergoes phase variation, or the ability to turn specific phenotypic encoded genes "on" and "off" depending on environmental factors (Kolenda et al., 2019). The curli fimbriae type is more commonly associated with *S. enterica* strains specifically and can be credited for the survival of toxin-producing cells in

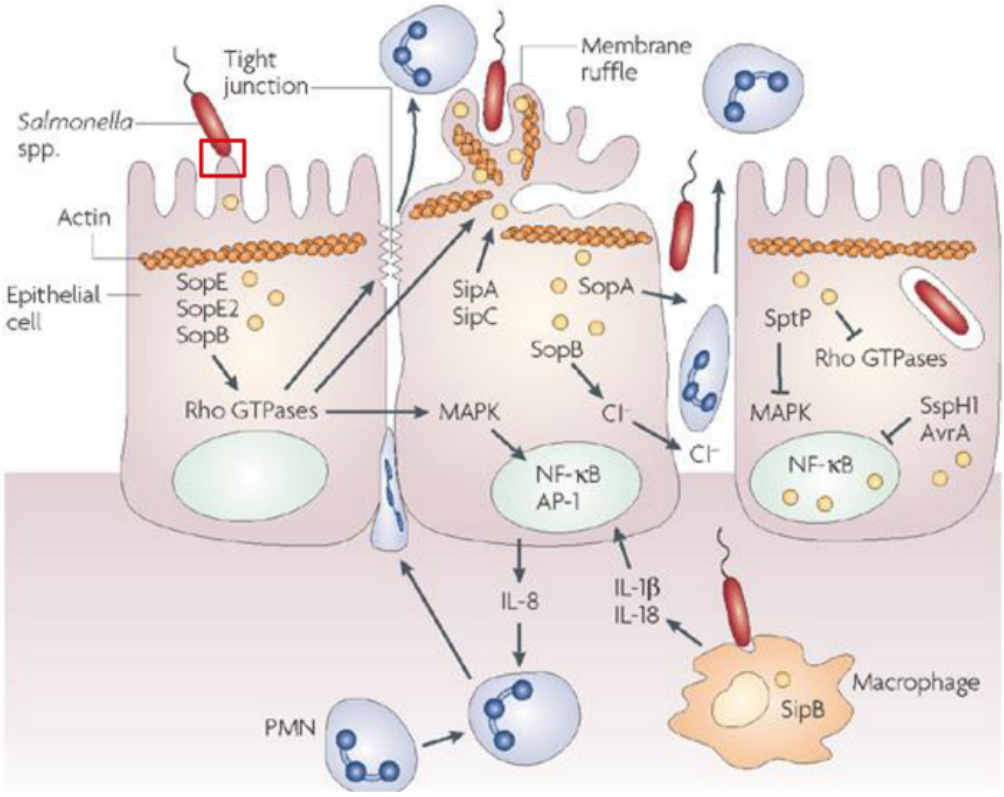

Figure 2.8: The internalization of Salmonellae (Haraga et al., 2008)

various environments, including the avian intestinal tract and non-biological hosts such as soil, because of its high relevance in biofilm formation (Rai & Bai, 2017).

The curli fimbriae type employs an attachment mechanism that binds to host-specific proteins, including fibronectin, laminin, plasminogen, and major histocompatibility complex class I molecules (Saldaña et al., 2009). Curli biogenesis in *Salmonella* is similar to *E. coli* and is mediated by the expression of the CsgD regulator of the csgBAC operon (Rai & Bai, 2017).

Once the Salmonellae have invaded the digestive tract, they attach to mannose-receptor residues within the intestine. This is when the shedding process begins, and further contamination occurs via fecal matter. These mechanisms are significant because of their contribution to the formation of the extracellular matrix needed for biofilm arrangement and allow for enhanced survival on both epithelial tissues and food contact surfaces.

2.9.2 Salmonella biofilms

A chapter published in the *Trends in Food Safety and Protection Journal* explains the role that biofilms play in the persistence of pathogenic bacteria and their contribution towards deadly outbreaks (Rai & Bai, 2017). A biofilm is a concentrated composition of bacteria that is encased in a sticky-like extracellular polymeric substance (EPS) matrix that allows resistance to environments that are not normally suitable for bacterial persistence (Rai & Bai, 2017). The EPS is composed of extracellular polysaccharides, proteins, extracellular DNA, and water (Zhao et al., 2023). This shield of polysaccharides allows for attachment to various surfaces, including both biotic and abiotic surfaces, as well as retains water and serves as a permeable membrane for gas emission and nutrient accumulation (Zhao et al., 2023). Because the accumulation of polysaccharides is so critical in biofilm formation, this mechanism could also take place in meat products during spoilage. Spoilage in any food results in an aggregation of nutrient compounds due to the degradation of protein tissues from enzymatic activity. Biofilm development is a key virulence mechanism for cross-contamination via food contact surfaces.

2.10 *Salmonella enterica* Prevalence in Raw Poultry Products

S. enterica has been identified as the current most prevalent bacteria causing foodborne illness, especially in poultry products (CDC, 2022, 2024). With the increasing demand of alternatively produced food products, the emergence of popularity for organic, antibiotic-free, free-range chicken, has outweighed the previous demand for conventional processes. Consumers believe that in unconventional processes, the lack of pesticides, antibiotics, vaccines, and added hormones increase the safety of poultry meat, however, there is a lack of scientific evidence to support this (Golden & Mishra, 2020; Sofos, 2008).

S. enterica contamination of poultry products can occur through both vertical and horizontal transmission (Foley et al., 2011). In poultry, vertical contamination at the farm can exist from breeding hens to offspring via transovarian contamination of the inner components of an egg. Eggshells can also be a source of contamination during the oviposition of the egg. The most common horizontal transmission of *S. enterica* in chickens is the fecal-oral route (Foley et al., 2011). *S. enterica* in poultry feces is considered reasonably likely to occur (RLTO) because of its nearly inevitable presence in broiler chicken houses (Chavez-Velado et al., 2024; Rai & Bai, 2017). According to the 2021 National Antimicrobial Resistance Monitoring System (NARMS) Integrated Report Summary, the top three serotypes among *S. enterica* isolated from retail chicken meat were Kentucky (38%), Enteritidis (23.3%) and Heidelberg (8.3%) (FDA, 2024).

A meta-analysis study by Golden & Mishra (2020) quantified the prevalence of *S. enterica* during three major benchmarks in the broiler chicken production chain. Results showed that the *Salmonella* prevalence was highest in prechill samples (68.6%), followed by rehang (42.9%), and postchill (14.3%). This data supports the claim that one of the most predominant

sources of bacterial contamination of poultry meat in poultry processing facilities occurs through transmission by processing equipment and food contact surfaces (Rouger et al., 2017).

Although prevalence of *Salmonella* can vary across processing plants based on their anti-microbial interventions such as peracetic acid washes and pH adjustments, a bio-mapping study identified consistently high areas of *S. enterica* detection across three plants (Chavez-Velado et al., 2024). The high areas mentioned were live receiving, rehang, and pre-chill (Chavez-Velado et al., 2024). Live receiving is the point of entry for birds to the facility; rehang is the step immediately after the scalding and picking process; prechill is before the immersion chilling step which remains the most significant critical control point in most operations due to the significant log reduction of bacterial counts from a mean concentration of 2.39 ± 0.23 log CFU/sample at live receiving to approximately 0.25 ±0.07 log CFU/sample during the post-chill step (Chavez-Velado et al., 2024). The comparison of *S. enterica* prevalence between these three areas can identify the strengths and weaknesses of the critical control points. A significant reduction of *Salmonella* prevalence from live hang to rehang may indicate that the scalding process between these two steps is effective at reducing microbial activity by the removal of feathers, head, feet and dirt from the carcass (Chavez-Velado et al., 2024). However, a higher percentage of *Salmonella* during the prechill step may indicate that cross-contamination of *Salmonella* positive carcasses is spread to *Salmonella* negative carcasses during the picking step due to the reuse of water and harborage of bacteria located within the picking fingers (Achtman et al., 2012; Chavez-Velado et al., 2024; Rouger et al., 2017). A significant portion of bacterial harborage can be attributed to cross contamination through contact with equipment surfaces, especially those with deep crevices, rough surfaces, and worn down or missing parts (Rouger et al., 2017).

The FSIS has regulations in place to control the prevalence of *Salmonella* during poultry slaughter and processing operations (FSIS, 2021). The Poultry Products Inspection Act (PPIA) establishes the mandating of continuous FSIS inspection during slaughter, sets sanitation and HACCP requirements, and regulates the labeling, handling, and facility operations of a poultry processing establishment. The regulations require facilities to conduct a hazard analysis during the development of a HACCP plan to determine the food safety hazards that occur at different steps of the processing line. During the development of a HACCP plan, critical control points are established to mitigate these hazards, such as post-chilling interventions. The frequency of *Salmonella* detection is regulated by the FSIS Performance Standards *Salmonella* Verification Program for Raw Poultry Products (FSIS, 2021). According to the *Salmonella* Performance Standards, following the chilling step, the performance guidelines permit a maximum percentage of positive *Salmonella* samples within a rolling 52-week window. In broiler carcasses the maximum allowable positives of *Salmonella* samples are 9.8%, 25% in comminuted meat, and 15.4% for chicken parts (FSIS, 2021). However, even with these performance standards, *Salmonella* infections continue to occur, suggesting that current regulations and mandated interventions may not be enough. Because of this, the Food Safety and Inspection Service (FSIS) proposed a new framework for *Salmonella* contamination levels in raw poultry products in August of 2024.

Although this framework was later withdrawn by the FSIS to further assess its approach on developing new regulations for the poultry industry, the reasoning for the proposal remains; to reduce the occurrence of *Salmonella* infections by 25% before the year 2030 (USDA, 2024). Products mentioned in the framework included raw chicken carcasses, chicken parts, comminuted chicken, and comminuted turkey products (FSIS, 2024). The proposed performance

standard included three major components of revision to current practices and standards enforced in poultry processing facilities (FSIS, 2024). While the first two components focused on the establishment of *Salmonella* as a hazard that is reasonably likely to occur (RLTO) in flocks before entering the facility and the required enforcement of microbial monitoring practices, the third component remains the most challenging (FSIS, 2024). This proposed component introduces a new final product detection threshold, under which a product would be considered adulterated if *Salmonella* levels exceed this limit or if specific high-risk serotypes are detected. This proposed standard stated that, " …raw chicken carcasses, chicken parts, comminuted chicken, and comminuted turkey are adulterated if they contain any type of *Salmonella* at or above 10 colony forming units/per milliliter or gram (10 cfu/mL(g)) in analytical portion (i.e., mL of rinsate or gram of product) and contain any detectable level of at least one of the *Salmonella* serotypes of public health significance identified for that commodity" (FSIS, 2024). While the implementation of a new regulatory standard may not have been the correct approach, the utilization of *Salmonella* monitoring and predictive tools is necessary to reduce these infection numbers.

2.11 Predictive Modeling

As the global population continues to rise, so does the demand for food (Quintieri et al., 2021). This creates pressure on the food industry to maintain an efficient food supply, while also engaging in sustainability practices that prevent overproduction and minimize unnecessary or preventable food waste. Challenge tests have been recognized as a standard application to determine the shelf life of products by simulating the effects of environmental conditions on food (McDonald & Sun, 1999). However, these tests can be considered economically and

operationally burdensome in terms of time, labor, and financial investment (McDonald & Sun, 1999). This makes them less suitable for rapid decision making. Predictive modeling is a statistical tool commonly used in the food industry to forecast outcomes of a product under specific conditions (McDonald & Sun, 1999; McMeekin et al., 1997; Neumeyer et al., 1997; Thomas et al., 2019). This can play a vital role in determining food safety, quality, shelf life, and assessing risk.

Predictive food microbiology (PFM) is a sector of predictive modeling that utilizes mathematical functions to develop a matrix that estimates the behavior of microorganisms' under different environmental conditions (McDonald & Sun, 1999; Ross et al., 2000; Thomas et al., 2019). Many food safety experts use this tool to predict the fate of a targeted bacteria under specific intrinsic or extrinsic conditions that may occur during processing, distribution, and storage of food products (Thomas et al., 2019). Models in food safety can be developed based on processing interventions that can determine the effectiveness of a critical control points in a HACCP plan or optimize the processing conditions for quality purposes. Food safety interventions include antimicrobial additions, temperature change, thermal processing, acidification etc. When deviations from product speculations occur, predictive microbiology can be used as an immediate application to estimate pathogen or bacterial growth, survival, or inactivation and help determine the condition of the product, whether it poses a food safety or quality-based risk (Ross et al., 2000).

Predictive microbial models can be categorized in different ways depending on the purpose and outcome. The most significant categories to note are: kinetic and probability-based, empirical and mechanistic, and primary, secondary, and tertiary models. Some of these categories

may overlap, thus adding to the complexity of the model, and proving that predictive modeling can serve many roles.

Kinetic models one of the primary approaches used in PFM that describe the rate of the bacterial behavior, such as growth or inactivation under various environmental conditions. This type of model quantifies the effect of environmental factors such as temperature, pH, and water activity on parameters such as lag time, specific growth rate, and population density (McMeekin et al., 1997; Van Boekel, 1996). These type of models are useful when estimating bacterial population or shelf-life over time and even under dynamic conditions (Baranyi & Roberts, 1994; Zwietering et al., 1991). In comparison, probability-based models estimate the probability of specific microbial activities occurring under given conditions (presence/absence)(McDonald & Sun, 1999; Ratkowsky & Ross, 1995). Probability models are most used to indicate the likelihood of growth or toxin production and not the speed of each (Roberts, 1997). A kinetic model is typically selected over probability-based models when the desired outcome requires precise quantification and speed of growth or inactivation. Traditionally, these two approaches have been considered as distinct, however, Ratkowsky and Ross (1995) proposed an integration of the two to better determine the boundary between growth/and no growth (Ratkowsky & Ross, 1995). They explained that the original division into the two categories was presumptive, and that eventually most research in PFM overlaps with both kinetic and probability-based approaches.

Empirical and mechanistic models describe the approach to predicting microbial behavior. Empirical models are built on data and designed to fit experimental data through mathematical relationships derived from microbial growth, survival, or inactivation, and do not define the biological processes such as enzymatic activity, nutrient metabolism, and stress

response that may be explained in mechanistic models (Devlieghere et al., 2009; McDonald & Sun, 1999; McMeekin et al., 1997; Zwietering et al., 1993). Mechanistic models focus on "theoretical bases" that govern cellular processes (McDonald & Sun, 1999). These models aim to represent the biological mechanisms that occur during cell growth or inactivation. Because of the significant impact that cellular metabolism has on the behavior of a bacterium, most researcher agree that mechanistic models provide more accurate and reflect the complexity of the microbial dynamics taking place (Jagannath & Tsuchido, 2003; Zwietering et al., 1993).

2.11.1 Primary Modeling

A primary model is used to define the distribution of microbial growth, survival, or death under isothermal, or constant conditions over time. A common primary model used for predictive microbiology is the Baranyi model (Figure 2.4). Figure 2.4 shows an example of the growth of *Listeria monocytogenes* under constant temperature conditions with the Baranyi model fitted (Thomas et al., 2019). It is important to select the most relevant type of primary model to a dataset based on the purpose and highest accuracy. The Baranyi model can be used to predict the growth, survival, and death curves. It is different from other primary models because of its accurate development of the adjusted sigmoidal curve by factoring in the separation and duration of different phases of bacterial growth: the lag phase, the exponential phase, and the stationary

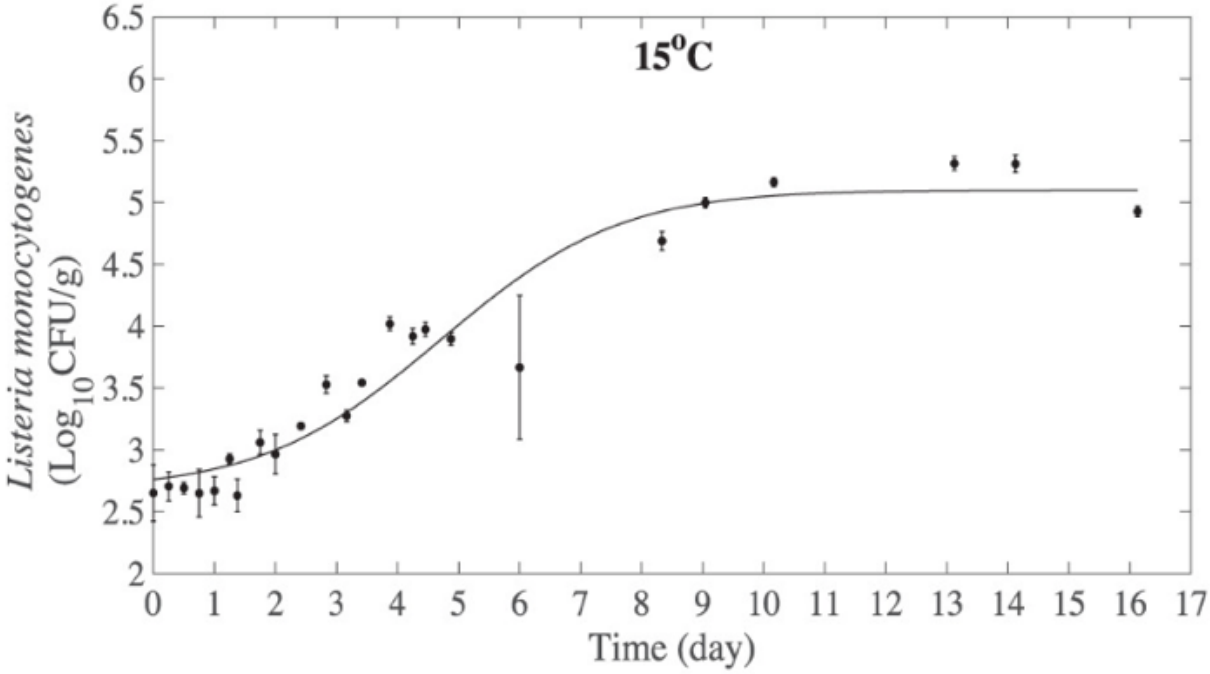

Figure 2.9: The Baranyi model used to show the growth of *Listeria monocytogenes* in queso fresco at a given temperature (Thomas et al., 2019)

phase. This model captures the time taken by bacterial cells to adjust to their environment, or the physiological adaptation referred to as the lag phase (Baranyi & Roberts, 1994; Roberts, 1997). There are several software's that utilize this model to help users to predict the bacterial concentration in each food product. Examples of this software include DMFit, Combase, or Pathogen Modeling Program (PMP).

2.11.2 Secondary Modeling

A secondary model is used to integrate the impact of the environmental parameter used on the primary model with a response to changes in one or more environmental factors such as temperature, pH, and water activity (a_w) (Baranyi & Roberts, 1994; Jagannath & Tsuchido, 2003; McDonald & Sun, 1999). The Ratkowsky square root model frequently provides an

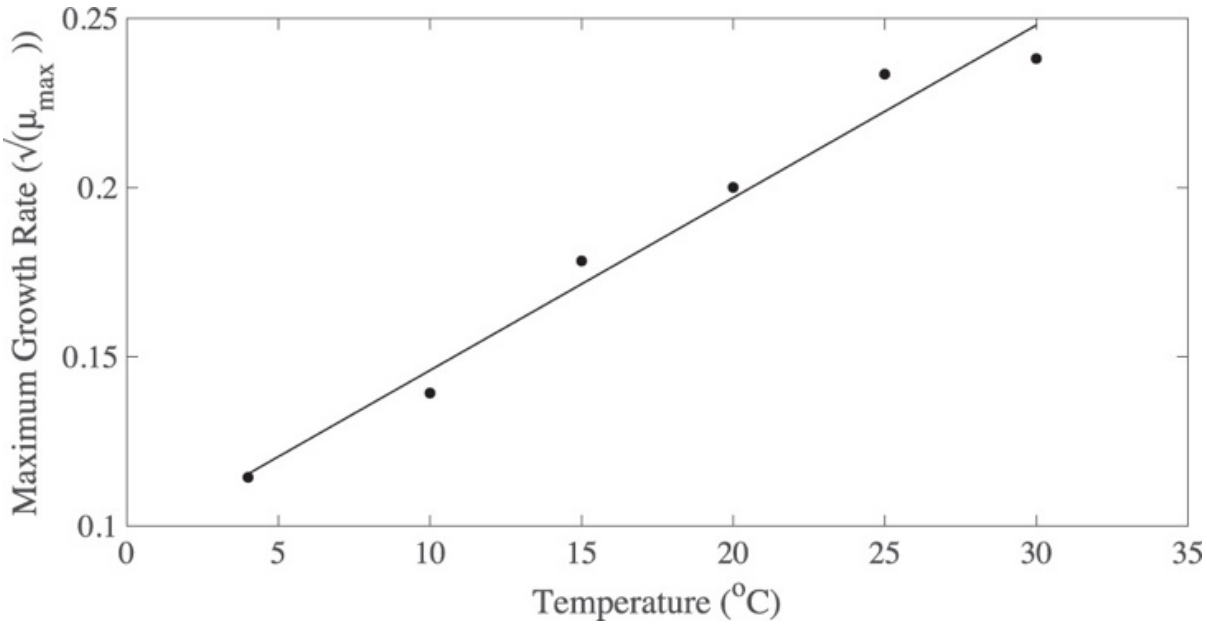

Figure 2.10: The Ratkowsky model used to fit the growth rate of *Listeria monocytogenes* in queso fresco at increasing temperatures (Thomas et al., 2019)

accurate fit during bacterial growth studies by interpreting the biological parameters (Ratkowsky et al., 1981; Thomas et al., 2019). Most models before the Ratkowsky model are based on the Arrhenius Law equation: $k = A \exp\left(-\frac{E}{RT}\right)$, which assumes microbial growth as exponential across all temperatures and interventions, including suboptimal conditions. Ratkowsky explained that bacterial growth is a complex process that the Arrhenius Law fails to describe, thus leading to his original model (Ratkowsky et al., 1981). Ratkowsky proposed a secondary model that employed a more accurate approach using square root as a function of bacterial growth and temperature: $\sqrt{\mu_{max}} = b(T - T_0)$ that provides an excellent fit to empirical data in both minimum and optimal growth temperatures (Ratkowsky et al., 1981). Later in 1983, Ratkowsky et al. extended the previously proposed Ratkowsky et al. (1981) model to cover the full biokinetic temperature range by including four new parameters that represent the minimum and

maximum temperature bounds (Ratkowsky et al., 1983). The new empirical non-linear regression model is as follows:

$$\sqrt{\mu_{max}} = b(T - T_{min})\{1 - \exp[c(T - T_{max})]\}$$

where T_{min} and T_{max} are the minimum and maximum temperatures, b is the regression coefficient of the square root of growth rate constant, and c is an additional parameter to better fit data for temperatures above the optimal range (Ratkowsky et al., 1983). Figure 2.5 shows an example of the current Ratkowsky model being used to show the distribution of data with an increasing growth rate of *Listeria monocytogenes* as temperature increases in queso fresco (Thomas et al., 2019).

2.11.3 Tertiary Modeling

Tertiary models are user-friendly computer software tools that are an integration of both primary and secondary modeling data. The most used software's include the USDA's Pathogen Modeling Program (PMP)(Buchanan, 1990) and the ComBase prediction system. This software allows users to predict microbial behavior under various environmental conditions and is supplied with data through validated and published models. Food safety professionals can easily access this database to predict the behavior of a specific bacteria under various conditions. Both programs allow for a quick simulation of microbial response to changing intrinsic and extrinsic factors such as temperature, pH, water activity, gas composition, etc.

2.12 Applications of Predictive Modeling

Predictive modeling has become a predominant tool in food microbiology by providing essential comprehensive approaches for predicting microbial behavior in varied food matrices under different environmental conditions. This mathematical application enhances food safety by enabling professionals to efficiently respond to high-risk events, develop and validate HACCP plans, determine shelf-life and spoilage capacity, and design processing interventions (McDonald & Sun, 1999). In meat and poultry products, modeling tools are being consistently developed and used due to the complex food matrix system and endless interventions used for animal-based protein products (Daud et al., 1978; Pooni & Mead, 1984). Data is constantly being added to modeling software to provide a more universal tool for food products that have been subjected to both unconventional and extensive processing operations.

During HACCP plan development and validation, food safety personnel can utilize the predictive modeling tool to accurately represent the behavior of bacteria at different points during their specific processing operation (McDonald & Sun, 1999). Predictive modeling can aid in quantifying the microbial population to allow professionals to adjust HACCP programs to comply with the thresholds set by the microbial performance standards. For example, the efficacy of an anti-microbial intervention for controlling spoilage bacteria such as *Pseudomonas* spp. in a poultry processing facility can be predetermined with a tertiary model. Such models can allow for the simulation of varying concentrations of peracetic acid under specific temperature and pH conditions. This application will eliminate excessive financial investment on unnecessary trial-and-error experimentation.

During risk-assessment of pathogen presence in a product exposed to temperature deviations, predictive modeling can be applied to rapidly evaluate the impact of the event and

accurately predict the fate of affected product. This application has the potential to reduce costs from product-recall and accelerate the risk assessment process that may otherwise be delayed during microbial testing of the product. The applications of predictive modeling in food safety continues to evolve, however, it is not without limitations.

2.13 Limitations of Predictive Modeling

While the applications of predictive microbiology span across multiple areas of both hazard analysis and quality assessment, certain constraints still limit the use of this modeling tool. Due to unpredictable behavior of bacteria exposed to atypical conditions, the need for microbial testing is essential. Industry users must provide empirical evidence for product holds and release decisions due to the high risk associated with contaminated product release. Additional limitations include the need for raw data to support model development in less common food categories (McDonald & Sun, 1999). Although popularity among predictive modeling utilization continues to rise, significant knowledge gaps persist for unconventionally processed foods (McDonald & Sun, 1999; Pooni & Mead, 1984). When new parameters need to be incorporated, the process of collecting data for model development is extensive and time consuming. Without the data to develop and validate a model, predictive modeling cannot be applied thus limiting its use to only well-documented food products. Another critical limitation of predictive microbiology is the overlooked-impact that competition has on microbial populations (Devlieghere et al., 2009). Bacteria can behave differently in the presence of a diverse bacterial population whereas certain species may outcompete others due to their adaptability and resilience in challenging conditions. This restricts the quantification process for the pathogen of concern and can potentially reduce the reliability of the model. As a result,

caution should be exercised when utilizing predictive modeling tools because of the inability to model unknown events that influence bacterial growth.

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Chapter 3

DEVELOPMENT AND VALIDATION OF A PREDICTIVE MODEL FOR *PSEUDOMONAS PUTIDA* IN RAW GROUND POULTRY MEAT

[1]Pollok, A. and Singh, M. To be submitted to *Journal of Food Protection*

3.1 Abstract

Pseudomonas spp. are a common spoilage organism and primary contributor to food quality degradation and food waste in poultry meat products due to its ubiquity, resilience during environmental stress, and psychrotrophic characteristics. This study sought to develop and validate a predictive growth model for *P. putida* in raw ground chicken meat under isothermal and dynamic temperature conditions, including the entire growth temperature threshold to mimic temperature fluctuations during transport and storage operations. *P. putida* was inoculated into irradiated raw ground chicken meat at seven isothermal temperatures (5, 10, 15, 20, 25, 30, 35°C) and quantified over time. The isothermal data was fitted with the Baranyi equation using the USDA-ARS Integrated Pathogen Modeling Program and yielded a low RMSE value ranging from 0.197-0.519, indicating a strong similarity between the predicted values and experimental data. The secondary model was fitted with the Ratkowsky Square Root equation to evaluate the effect of temperature on maximum specific growth rate (μ_{max}) and evaluated with an RMSE value of 0.049, suggesting a high degree of conformity between the observed values and prediction. The tertiary model was formulated through the synthesis of the data obtained from the primary and secondary models and validated using two dynamic sinusoidal temperature profiles: 5-20°C, 24-hour cycle*;* 15-35°C, 6-hour cycles. Model performance was assessed using Accuracy (Af) and Bias (Bf) factors for both profiles: Af (0.0441) and Bf (1.0260) for the low temperatures, and Af (1.050) and Bf (0.9815) for the high temperatures. These findings suggest that the developed prediction model exhibited close alignment with the observed data, indicating a reliable model for industry use in predicting the bacterial behavior of *P. putida* in raw ground chicken meat subjected to dynamic temperature conditions.

3.2 Introduction

Pseudomonas spp. are ubiquitous Gram-negative, facultative anaerobes that are commonly found in soil, water, and areas of high humidity. Although foodborne isolates of this organism are not typically associated with illness or raise concern as a serious health threat like some pathogenic strains (Mellor et al., 2011), it is most commonly implicated in the spoilage of animal-based protein products such as eggs, dairy, and meat. This can be attributed to its ability to persist under challenging processing conditions, psychrotrophic characteristics, and lipolytic and proteolytic enzymatic activity (Neumeyer et al., 1997). *Pseudomonas* frequently outcompetes other bacteria due to these resilient properties (Wickramasinghe et al., 2019). *Pseudomonas* spp. and other spoilage organisms can serve as indicators of poor sanitation and are not regulated in the food industry; however, the United States Department of Agriculture (USDA) has regulations that require the development, and validation of written sanitation and hygiene protocols such as Good Manufacturing Practices (GMPs) and Sanitation Standard Operating Procedures (SSOPs) that target the minimization of microbial growth.

Pseudomonas spp. are recognized as the primary spoilage organism found in poultry meat products, and other animal-derived protein foods (Heir et al., 2021; Hinton et al., 2004; Koutsoumanis et al., 2006). Its ability to withstand intensive processing efforts through multiple survival mechanisms makes it a key contributor to the degradation of sensory quality in meat products (Quintieri et al., 2021; Thomassen et al., 2023). Among poultry cuts, skin-on and comminuted products are prone to increased spoilage rates (Hinton et al., 2004). Skin-on poultry retain higher bacterial loads due to their feather follicles and water retention, which can increase bacterial growth (Dawson et al., 2013; FSIS, 2025). Food contact surfaces and equipment are also a common reservoir for bacterial contamination. Frequent sources of contamination from

equipment include the rubber picking fingers used for feather removal and conveyor belts (Rouger et al., 2017), which contain pronounced crevices that create ideal niches for bacterial harborage and biofilm development (Arnold & Yates, 2009; Rouger et al., 2017). Additional sources of contamination along the poultry process include airborne bacteria, water from scalding and frequent rinsing and chilling steps, cross-contamination during evisceration, deboning, cutting, mincing, and ingredient additions during further processing (Rouger et al., 2017). Given the numerous potential entry points for spoilage bacteria throughout poultry processing, their presence is inevitable, posing a substantial economic concern.

According to the USDA, food waste is defined as edible food products that are not eaten and discarded (Buzby et al., 2014). In 2010, approximately 30-40 percent of the United States' food supply was lost as waste, totaling an estimated weight of 133 billion pounds and representing a financial loss of $161 billion (Buzby et al., 2014). The Economic Research Service (ERS) estimated that in 2010, of the 22 billion pounds of poultry meat produced, 21.8% was lost to waste, including microbial spoilage and contamination (Buzby et al., 2014). While food waste can accumulate from various stages throughout production, spoilage remains a leading contributor. Food waste not only results in the loss of edible nutrients and financial investment in the product but also represents a waste of resources invested in the production, including land, water, labor, and energy. The ERS uses the term "negative externalities" to account for these indirect economic losses accumulated from unintentional consequences of one action that impose on the environment and societal costs (Buzby et al., 2014).

As the global population continues to rise, the demand for food increases, and the pressure on the food industry accumulates (Quintieri et al., 2021). The food industry is responsible for ensuring a stable food supply while also implementing sustainable production

practices to reduce waste. Conventional methods of evaluating product shelf life are the use of challenge tests, which simulate environmental storage and transportation conditions that affect the shelf life of a product (McDonald & Sun, 1999). Although effective, these methods are time-consuming, labor-intensive, and costly, making them impractical for rapid decision making (McDonald & Sun, 1999). Predictive modeling is a widely used alternative that allows for a more efficient approach, and this approach uses statistical functions to estimate the behavior of bacteria under specific environmental conditions, aiding in the risk assessment of food safety concerns, deviations from product speculations, and shelf life determination (McDonald & Sun, 1999; McMeekin et al., 1997; Neumeyer et al., 1997; Thomas et al., 2019).

A specific sector for applications of this tool is Predictive Food Microbiology (PFM), which enables food safety professionals to simulate microbial behavior, such as growth, inactivation, and survival, under various environmental conditions that can affect a food product (McDonald & Sun, 1999; Ross et al., 2000; Thomas et al., 2019). The objective of this study was to determine the minimum and maximum growth temperatures for *Pseudomonas putida* in raw ground poultry meat, develop a predictive model for the growth of *P. putida* under a full growth temperature profile (5 to 35 °C), and to validate the developed model by using a high and low temperature profile under sinusoidal temperature fluctuations.

3.3 Materials and Methods

3.3.1 Ground Chicken Meat

Poultry meat breasts and thighs cuts were obtained from a local grocery store and further processed using equipment in the food processing pilot plants in the Department of Food Science and Technology at the University of Georgia. The meat was ground twice through a fine grind (1/8mm grind plate). The ground chicken was then packaged (8" x 10", Clarity 3 mil Standard Bags, Clarity Brand) and vacuum sealed with approximately 120g each prior to being frozen. The frozen bags were then packaged in perishable shipping kits and sent off for electron beam irradiation at the National Center for Electron Beam Research at Texas A&M University in College Station, Texas. The bags were irradiated with 3.5 kGy for total degradation of all bacteria, making them sterile for experimental inoculation. The meat was then stored at -20 °C before being thawed at 4 °C, 12 h prior to use. Following irradiation, various random samples were used to measure the average pH, 6.06 ± 0.06. The fat concentration was measured as 3.17 ±17 by the ANKOM^{XT15} Extraction System (ANKOM Technology, Macedon, NY) at the University of Georgia Department of Animal and Dairy Science.

3.3.2 Bacterial Strains and Inoculation

Pseudomonas putida isolate used in this study was obtained from the American Type Culture Collection (ATTC, Manassas, VA). The strain was cultured from the glycerol stocks in the -80 °C storage in two biological replicates. A 10uL loop of the stock was used to inoculate a sterile test tube with 10mL of Tryptic Soy Broth (TSB; Difco, Sparks, MD) and grown at 30°C for 18 h to obtain an inoculum population of ~ 8.5-9.0 log CFU/mL. The inoculum was further

diluted to target a starting population of 2.5-3.0 log CFU/g in the meat pouches across all replicates and temperature experiments. The product was inoculated and divided into 5 g aliquots. The ground poultry pouches were then heat-sealed and homogenized by hand massaging for two min.. Small pouches of meat were made with 5 ± 0.10g of inoculated meat and heat sealed. The pouches were then submerged in a circulating water bath (PolyScience, Cole-Parmer Instrument Company, Niles, IL) at a constant temperature of 5, 10, 15, 20, 25, 30, and 35°C and held throughout the study.

3.3.3 Sampling

At the predetermined fixed time points for sampling, a pair of replicate samples were removed from the water bath, and the meat from the pouches was aseptically transferred into filtered Whirl-Pak bags (Nasco International, Madison, WI) with 20mL of 0.1% Buffered Peptone Water (BPW; Difco, Stark, MD). The pouches were then hand massaged for two min., and a series of tenfold serial dilutions were prepared in 0.1% BPW. The selected dilutions were then spread plated in duplicates on *Pseudomonas* Isolation Agar (PIA; Sigma-Aldrich, St. Louis, MO) in 100uL aliquots and incubated for 24 h at 30°C.

3.3.4 Predictive Modeling

Primary Model

A primary model is used to define the distribution of how a microbial population grows, survives, or dies under isothermal, or constant conditions, over time. It is important to select the

most relevant primary model for a dataset based on the purpose and highest accuracy. A common primary model used for predictive microbiology is the Baranyi model. The Baranyi model can be used to predict the growth, survival, and death curves, and is different from other primary models because of its accurate development of the adjusted sigmoidal curve by factoring in the separation and duration of different phases of bacterial growth: the lag phase, the exponential phase, and the stationary phase. This model captures the time taken by bacterial cells to adjust to their environment, or the physiological adaptation referred to as the lag phase (Thomas et al., 2019). Once isothermal data was collected from each temperature set, the regression curve from the bacterial cell populations (log CFU/mL) at given time points was fitted to the primary modeling equation with the USDA Integrated Pathogen Modeling Program 2013 (IPMP) software and was used for developing the primary and secondary predictive models in the present study. This program can allow users to analyze the behavior of bacteria during growth, survival, or inactivation under various environmental changes such as temperature, pH, water activity (Aw), etc. The equation for the fitted model is as follows:

Baranyi Model equation:

$$y(t) = y_0 + \mu_{max}F(t) - \ln\,(1 + (\frac{e^{\mu_{max}F(t)} - 1}{e^{(y_{max} - y_0)}})$$

where $F(t) = t + \frac{1}{v}\ln\left(e^{-vt} + e^{h0} - e^{(vt-h0)}\right)$. The parameters of the equation are defined as: $y(t)$is the cell population of *Pseudomonas putida* (log CFU/g) at time t, $y_0$is the initial cell population (log CFU/g), $y_{max}$is the maximum cell population reached (log CFU/g), μ_{max} is the specific growth rate (log CFU/h), h_0 describes the prior physical state of the cells. The lag phase

duration was calculated using the formula: $\lambda = \frac{h_0}{\mu_{max}}$ where λ is the parameter for lag phase duration (h) (Zwietering et al., 1991).

Secondary Modeling

A secondary model is used to integrate the impact of the environmental parameter used in the primary model. The Ratkowsky square root model frequently provides an accurate fit during bacterial growth studies by interpreting the biological parameters. The parameters given from the primary modeling output were used to develop a secondary model to demonstrate the relationship of temperature and specific growth rate (μ_{max}). The values from each replicate were fitted into the secondary Ratkowsky Square Root model (Ratkowsky et al., 1981). The equation is as follows:

$$\sqrt{\mu_{max}} = b(T - T_{min})\{1 - exp[c(T - T_{max})]\}$$

where the a parameter is the regression coefficient, T is the temperature (°C), and T_{min} is the minimum temperature (°C).

Tertiary Model

Tertiary models are user-friendly software and allow for the accurate prediction of bacterial behavior under specific conditions. The role of a tertiary model is to simulate real-world temperature fluctuations that may occur during transportation and storage. This type of model is developed by the integration of primary and secondary models that have been validated with sinusoidal dynamic conditions. Such software includes that of the most widely used, IPMP software (Huang, 2013). However, a major limitation of this software is that it is restricted to accessing raw data. Another common USDA-ARS software applied is ComBase (Barayni &

Tamplin, 2004), which includes access to a database with over 65,000 records that describe the behavior of pathogens and spoilage organisms in a diverse food system. The software used for the development of the tertiary model in this study was MATLAB (Version R2023a, The MathWorks, Inc). This program allowed for the precise input of experimental parameters and conditions to predict the growth of *P. putida* accurately.

3.4 Statistical Analysis

The statistical analysis of the primary models included the comparison of goodness-of-fit metrics, including Root Mean Squared Error (RMSE), Sum of Squared Errors (SSE), Mean Squared Error (MSE), and Residual Standard Deviation. These values were given in the statistical output following the fitting of the Baranyi model to the experimental data using the USDA-ARS Integrated Pathogen Modeling Program software. The formulas for each metric are displayed in Table 3.1. The analysis of the secondary model included the evaluation of RMSE, while also identifying trends that can indicate normal bacterial behavior observed during growth, such as maximum specific growth rate (μ_{max}) and lag phase duration (LPD). The tertiary model fitness was evaluated with the Accuracy and Bias factors calculated from the MATLAB software (Baranyi et al., 1999).

3.4.1 Validation of Models

The tertiary predictive growth model was validated by conducting two dynamic sinusoidal time-temperature profiles under constant intrinsic conditions: 5-20 °C (24-hour cycle); 15-35 °C (6-hour cycles). As previously mentioned, inoculated samples were placed in a circulating programmable water bath to simulate temperature fluctuations at fixed intervals.

Sampling was conducted at planned time intervals, and the growth of *Pseudomonas putida* was enumerated until cells reached the stationary phase.

3.5 Results and Discussion

3.5.1 Temperature threshold

Pseudomonas putida exhibited growth in ground poultry meat under constant isothermal conditions from temperatures 5 to 35°C. The minimum and maximum temperatures for bacterial growth were tested through preliminary studies. For the lower limit, the bacterial concentration of *Pseudomonas putida* did not detect growth at temperatures lower than 5°C, but survival was indicated. The limit of detection (LOD) for *P. putida* was 0.84 log CFU/g. Although *Pseudomonas* spp. are considered a psychrotroph, this strain of bacteria did not exhibit growth below the typical threshold of 4-5°C likely due to severely reduced metabolic activity (Barbier et al., 2014). For the upper limit, *P. putida* growth was not detected at temperatures greater than 35°C, and death of the bacterial cells was indicated at a temperature of 37°C (LOD = 0.84).

3.5.2 Primary Modeling

This study measured the growth of *Pseudomonas putida* at seven different temperatures: 5, 10, 15, 20, 25, 30, and 35°C. The bacterial growth was from each of these isothermal experiments was enumerated, converted to log CFU/g, and fitted with the Baranyi equation using the USDA Integrated Pathogen Modeling Program 2013 (IPMP) software (Figure 3.1). The growth parameters: maximum specific growth rate (μ_{max}), lag phase duration (LPD, λ), initial cell

concentration (y0), and maximum cell population (y_{max}) were recorded (Table 3.2). The parameter h0 is characterized as the 'adaptation work' required of a bacteria to adapt existing conditions (Juneja et al., 2007; Thomas et al., 2019; Tsaloumi et al., 2022). It is assumed in the Baranyi equation that the value of h0 is constant for a given bacterial species in a specific food matrix (Baranyi & Roberts, 1994; Juneja et al., 2007; Thomas et al., 2019). However, it was determined in this study that there was no correlation between temperature and h0 values, therefore, the arithmetic mean of h0 (1.07) was used for further modeling development. Because of this lack of pattern, the primary models were refitted with the new h0 value, and the other parameters were re-calculated. The isothermal enumeration and predicted curve for each temperature is shown in Figure 3.1.

The initial cell concentration (y0) of the inoculated meat pouches ranged from 2.48-3.53 log CFU/g across all temperatures compared to the ideal target inoculation of 2.5 log CFU/g (Table 3.2). The maximum population density detected did not exceed 8.09 log CFU/g during any of the isothermal experiments. This could be attributed to the limited amount of oxygen and nutrients available to the bacteria because of the impermeable pouch material that is traditionally used for vacuum sealing.

While goodness of fit metrics are recommended to be utilized relatively and not as an absolute standard, these values can provide insight into the performance of a model. The equations associated with each metric can be found in Table 3.1. Overall, the fitting of the observed primary isothermal data to the Baranyi model is reflected with low Root Mean Squared Error (RMSE) values ranging from 0.197-0.519 (Table 3.3). During RMSE analysis, the closer the value is to zero, the "better fit" a model is to the observed data. These values indicate that the observed data was very similar to the model predictions. The Sum of Squared Error (SSE) is also

given as a comparative metric to use when multiple model types are fitted. However, since only the Baranyi model was fitted and the value of SSE increases with sample size, this metric was not used to assess the goodness of fit in the present study, but it can be used for future studies comparing additional fitted model equations (Table 3.3). Another metric extracted from the modeling software was the Mean Squared Error (MSE) values that ranged from 0.070-0.384. This metric reports values as squared units compared to RMSE which has the units used in the observed model data. MSE values are also used as another alternative comparison metric for model developers to use to evaluate the optimization of a model and are not as relevant in the present study but are provided for future research purposes.

3.5.3 Secondary Modeling

The maximum specific growth rate (μ_{max}) showed a positive correlation as it increased with increasing temperatures up to its optimal growth temperature of 30°C, then showed a slight decrease at 35°C (Figure 3.2) When fitted with the Ratkowsky model, the observed values showed adherence to the predicted values with and resulted in an RMSE value of 0.049, indicating the model was accurate. The Lag Phase Duration (LPD) is the amount of time for bacterial cells to physiologically adapt to the environment. The LPD of *Pseudomonas putida* in ground poultry meat was observed to be negatively exponential as temperature increased, reaching its minimum length at its optimal growth temperature of 30°C, after which it began to increase again (Figure 3.3). Baranyi et al. (1995) explains that LPD can be a function of temperature, and exposure to temperatures near the lower limit of an organisms growth range can induce cold shock that can result in a prolonged lag phase (Baranyi et al., 1995). This claim is

supported by the data observed in the present study. The Ratkowsky reported that the predicted minimum temperature (T_{min}) for growth was 6.01 ± 1.69°C and the predicted maximum growth temperature (T_{max}) was 35.32 ±0.01°C for *P. putida* in raw ground poultry meat. These values remain close to the observed minimum (5°C) and maximum (35°C) temperatures.

3.5.4 Growth Models Under Dynamic Temperature Profiles and Validation Evaluation

Figure 3.4 displays the predicted tertiary model profiles developed from MATLAB software, and the observed data from the validation studies. The lower temperature profile (5-20°C) yielded an Accuracy factor (Af) of 1.0441 and a Bias factor (Bf) of 1.0260. The higher temperature profile (15-35°C) yielded an Accuracy factor of 1.050 and Bias factor of 0.9815. The role of the Accuracy factor is to reflect the closeness of the predicted values sourced from the tertiary model to the observed values obtained from the validation experiment by measuring the deviation(Baranyi et al., 1999). Bias factor indicates whether there is an over or under-prediction, if any, of the bacterial behavior (Baranyi et al., 1999). For both parameters, a value approaching 1.0 reflects the greater reliability and applicability of the model (Baranyi et al., 1999). For the present study, the factor values suggest the tertiary models are well-fitted to the prediction of the bacterial growth of *Pseudomonas putida* in raw ground poultry meat. An Accuracy Prediction Zone (APZ) analysis was performed to further assess any deviation between the observed and predicted data (Figure 3.5). This was achieved by subtracting the observed values from the predicted values to generate the predicted error. The acceptable prediction limit was set between -1.0 (fail-safe) and 0.5 (fail-dangerous) log CFU/g. It is important to note that when predicting the bacterial behavior within a food matrix, some deviations are to be expected.

A fail-safe model can indicate the overprediction of growth, and a fail-dangerous model can indicate an underprediction of growth (Juneja et al., 2018; Oscar, 2005; Thomas et al., 2019). This analysis yielded an APZ score of 96.16% with only one out of 26 points from both temperature profiles falling outside of the accepted accuracy prediction zone (-1.0 to 0.5 log CFU/g). An APZ score of >70% is preferred and considered as an acceptable score (Oscar, 2005). This indicates a high level of agreement between the observed and predicted values, thus demonstrating a strong model performance under dynamic conditions.

3.6 Conclusion

This study successful characterized the temperature-dependent growth of *Pseudomonas putida* in raw ground poultry meat using predictive modeling approaches. This organism demonstrated growth between 5°C and 35°C, with no detectable growth below 5°C or above 35°C. Primary modeling using the Baranyi equation yielded low RMSE values (0.197-0.519), indicating a strong fit between observed and predicted growth under isothermal conditions. Secondary modeling with the Ratkowsky equation confirmed the positive relationship between temperature and maximum specific growth rate, up until its optimal growth temperature of 30°C. The lag phase duration exhibited a negatively exponential relationship as temperature increased. The validated tertiary models demonstrated high predictive reliability, with accuracy and bias factors close to 1.0 for both low and high temperature profiles. The Accuracy Prediction Zone (APZ) analysis yielded a score of 96.16%, well above the threshold of 70% acceptability. Overall, these results prove that this model can serve as an effective tool for predicting the growth behavior of *P. putida* under dynamic temperature conditions in raw ground poultry meat

and can offer valuable insights in shelf-life determination, quality control risk assessment, and spoilage prevention.

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LIST OF FIGURES

Figure 3.1

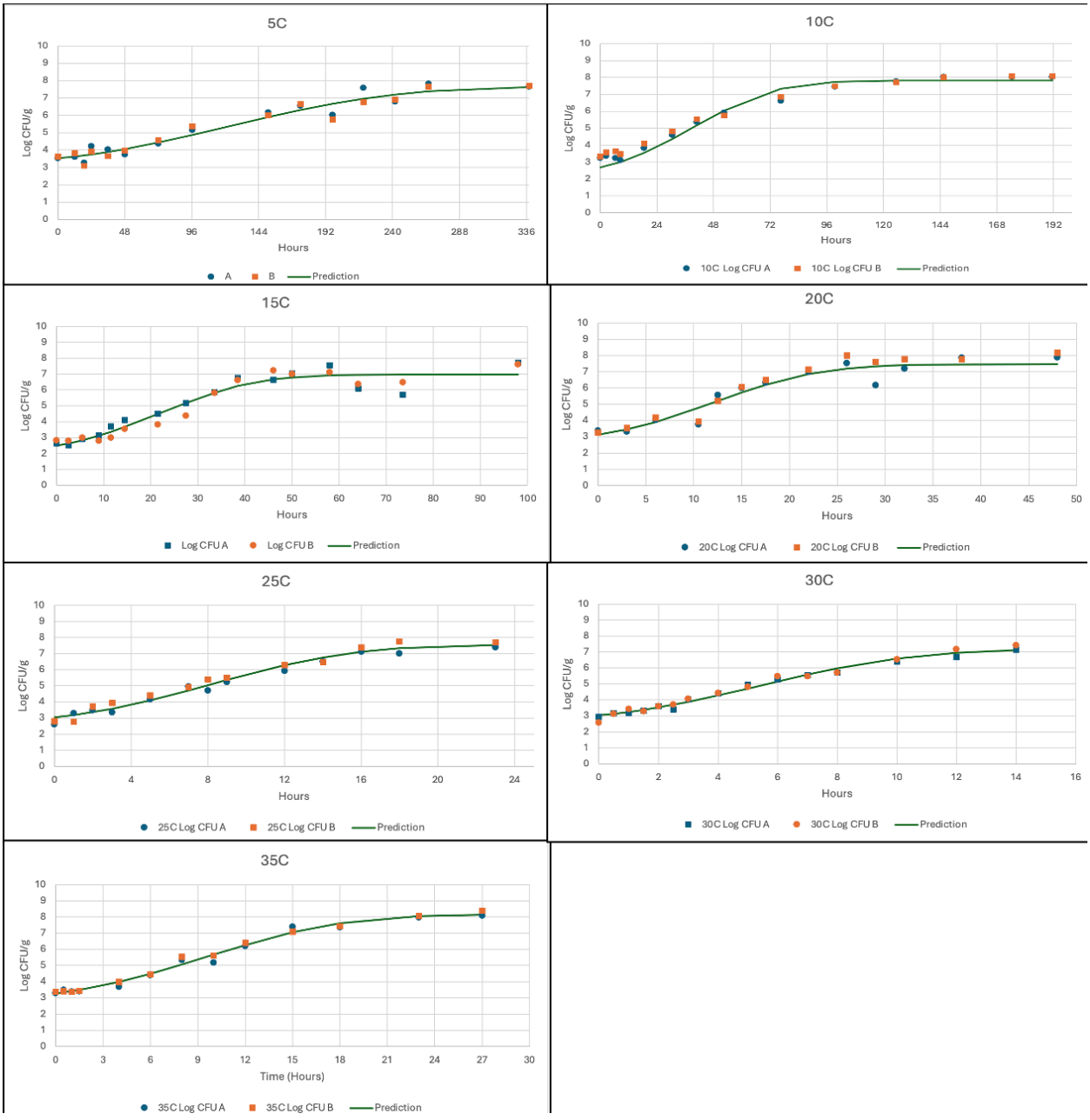
5C
Log CFU/g
Hours
A
B
Prediction
10C
Log CFU/g
Hours
10C Log CFU A
10C Log CFU B
Prediction
15C
Log CFU/g
Hours
Log CFU A
Log CFU B
Prediction
20C
Log CFU/g
Hours
20C Log CFU A
20C Log CFU B
Prediction
25C
Log CFU/g
Hours
25C Log CFU A
25C Log CFU B
Prediction
30C
Log CFU/g
Hours
30C Log CFU A
30C Log CFU B
Prediction
35C
Log CFU/g
Time (Hours)
35C Log CFU A
35C Log CFU B
Prediction

Figure 3.2

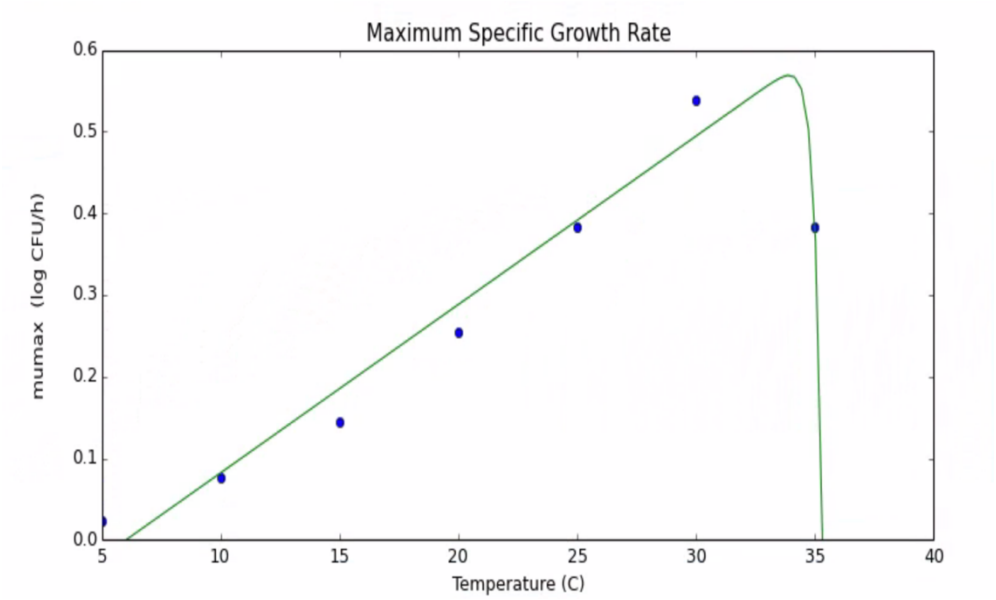
Maximum Specific Growth Rate
mumax (log CFU/h)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
5
10
15
20
25
30
35
40
Temperature (C)

Figure 3.3

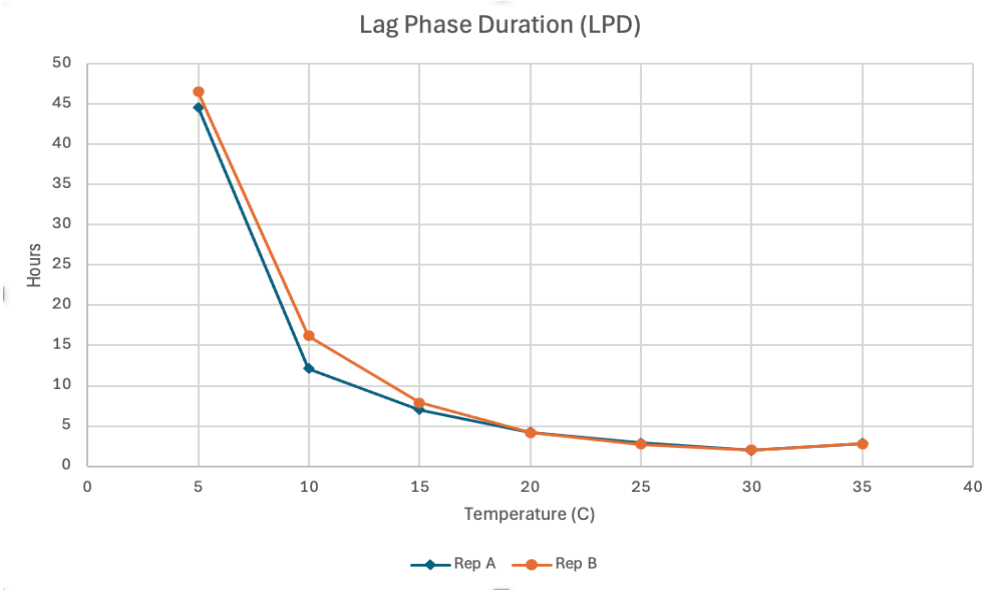
Lag Phase Duration (LPD)
Hours
50
45
40
35
30
25
20
15
10
5
0
0
5
10
15
20
25
30
35
40
Temperature (C)
Rep A
Rep B

Figure 3.4

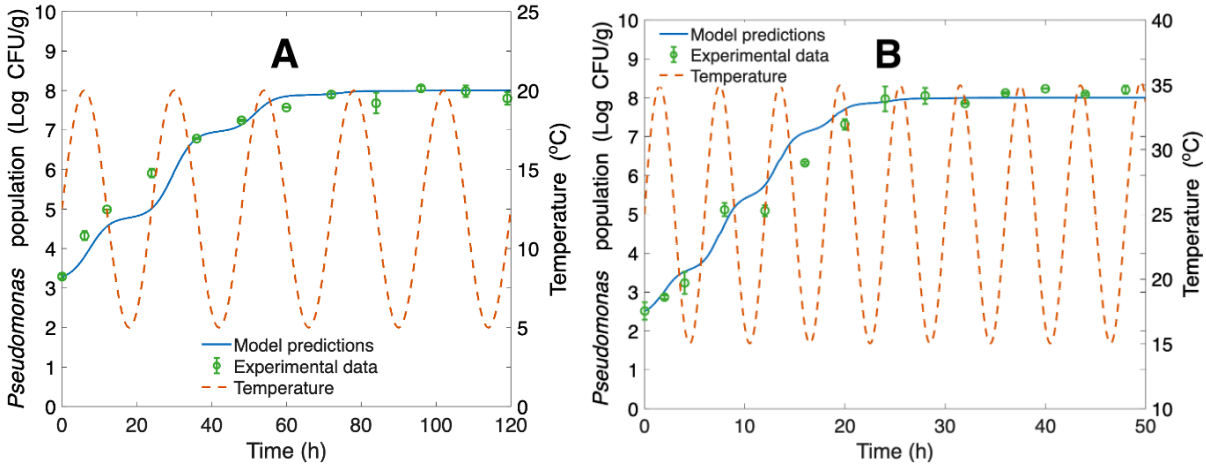
A
B
Model predictions
Experimental data
Temperature
Pseudomonas population (Log CFU/g)
Temperature (°C)
Time (h)

Figure 3.5

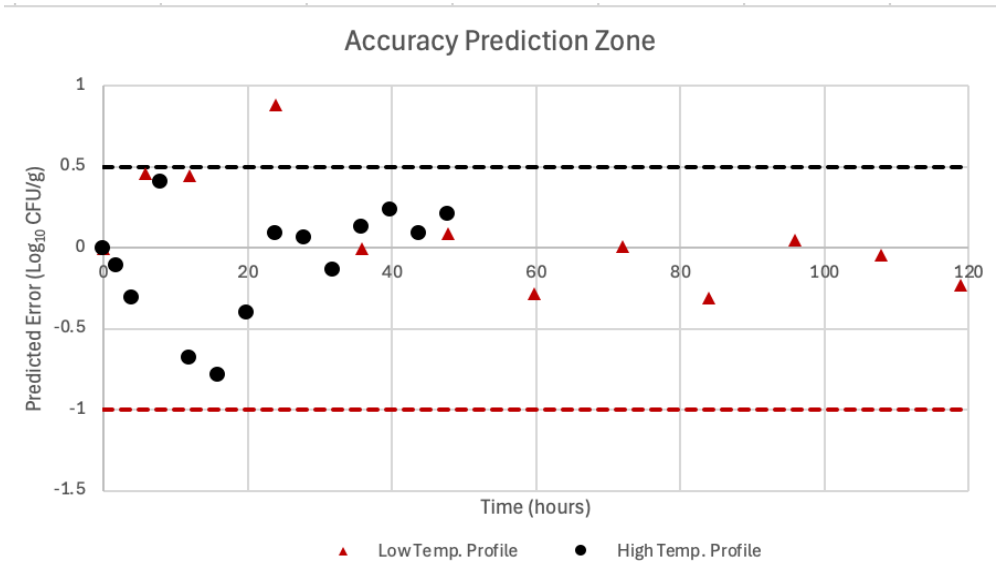
Accuracy Prediction Zone
Predicted Error (Log10 CFU/g)
1
0.5
0
-0.5
-1
-1.5
0
20
40
60
80
100
120
Time (hours)
Low Temp. Profile
High Temp. Profile

Table 3.1: Goodness of fit metrics and the associated formulas

Metric	Name	Formula
SSE	Sum of Squared Errors	$\sum_{i=1}^{n}(y_i - \hat{y}_i)^2$
MSE	Mean Squared Error	$\frac{1}{n}\sum_{i=1}^{n}(y_i - \hat{y}_i)^2$
RMSE	Root Mean Squared Error	$\sqrt{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}$
RESIDUAL SD	Residual Standard Deviation	$\sqrt{\frac{SSE}{n-p}}$

Table 3.2: Specific growth rate(μ_{max}), maximum population density (y_{max}), lag-phase duration (λ), and initial bacterial concentration (y0) of *P. putida* in raw ground poultry meat at different temperatures.

Temp (°C)	μmax (log CFU/h)	y_{max} (log cfu/g)	λ (h)	y0(log cfu/g)
5	0.02	7.70	45.55	3.53
10	0.08	7.88	14.18	3.02
15	0.14	6.98	7.45	2.48
20	0.25	7.74	4.21	3.13
25	0.38	7.57	2.79	3.03
30	0.54	7.22	0.53	3.03
35	0.38	8.09	0.38	3.22

Table 3.3: Primary modeling Goodness of Fit Metrics extracted from the USDA Pathogen Modeling Program Software for *P. putida*

Temp (ºC)	RMSE	SSE	MSE	RESIDUAL SD
5	0.40	1.89	0.16	0.35
10	0.52	4.59	0.38	0.46
15	0.50	3.45	0.30	0.46
20	0.46	2.25	0.22	0.41
25	0.26	0.71	0.07	0.23
30	0.20	0.47	0.04	0.18
35	0.32	1.26	0.11	0.29

Chapter 4

DEVELOPMENT AND VALIDATION OF A PREDICTIVE MODEL FOR *SALMONELLA ENTERICA* IN RAW GROUND POULTRY MEAT

[1]

[1] Pollok, A. and Singh, M. To be submitted to *Journal of Food Protection*

4.1 Abstract

Salmonella enterica is a leading bacterial pathogen associated with foodborne illness in the United States, with the consumption of contaminated poultry products contributing to 23% of the 1.35 million annual infections. Despite extensive interventions in the poultry industry to mitigate contamination, *Salmonella* remains prevalent and is considered reasonably likely to occur (RLTO) during poultry processing. The present study aimed to develop and validate a predictive growth model for *Salmonella enterica* in raw ground poultry meat under isothermal and dynamic temperature conditions, including the entire growth temperature threshold to mimic temperature fluctuations during transport and storage operations. A five-strain cocktail of *Salmonella enterica* serovars (Hadar, Heidelberg, Montevideo, Thompson, and Enteritidis) was inoculated into irradiated raw ground chicken meat and stored at nine isothermal temperatures (7, 10, 15, 20, 25, 30, 35, 40, 45 °C). Bacterial growth was enumerated over time and fitted with the Baranyi primary model using the USDA-ARS Integrated Pathogen Modeling program (IPMP 2013). This resulted in RMSE values ranging from 0.065 to 0.303, supporting the indication of close alignment between the observed values and the predicted outcome. The specific growth rate (μ_{max}) was used to develop the secondary model. A Ratkowsky square root equation was used for secondary modeling to describe the effect of temperature on μ_{max}, resulting in a strong fit yielding an RMSE value of 0.083. The tertiary model was developed by integrating the primary and secondary models and validated under two sinusoidal dynamic temperature profiles: 7–25 °C with 12 h cycles and 20–45 °C with 8 h cycles. The performance of the low and high models was evaluated using Accuracy (0.9771, 1.034) and Bias (1.044, 1.012) factors, respectively, which indicated strong agreement between the predicted values and experimental data during validation studies. These results indicate that the developed model is closely related

to the observed data, demonstrating its reliable application for predicting the growth behavior of *Salmonella enterica* in raw ground chicken meat under dynamic temperature conditions.

4.2 Introduction

Salmonella is a Gram-negative facultative anaerobe and is the leading bacterial pathogen associated with foodborne illness (FDA, 2024). The U.S. Centers for Disease Control and Prevention (CDC) estimates that *Salmonella* causes approximately 1.35 million infections in the United States every year, with nearly 23% of cases attributed to the consumption of contaminated poultry products (Lamichhane et al., 2024; Scallan Walter et al., 2025). Salmonellosis, a bacterial disease caused by *Salmonella*, can result from consuming contaminated food products that may lead to a range of symptoms, including gastrointestinal disease and, in extreme cases, death (Lamichhane et al., 2024). Despite substantial research efforts and industry interventions to minimize the presence of *Salmonella* in poultry meat, this pathogen remains prevalent in raw poultry products, warranting its categorization as "Reasonably Likely To Occur" (RLTO) (Chavez-Velado et al., 2024; Rai & Bai, 2017).

Beyond health implications, the economic burden associated with *Salmonella* contamination is substantial. Scharff (2020) estimates that the total cost of *Salmonella* in poultry meat equates to an estimated cost of $2.8 billion in health issues, product waste, decline of life expectancy, etc. (Scharff, 2020). The staggering losses highlight the need for more effective control strategies and regulatory compliance. Recognizing the importance of reducing the burden of salmonellosis, the U.S. Department of Agriculture's Food Safety Inspection Service (USDA-FSIS) prioritized the reduction of *Salmonella*-related illnesses by proposing a new *Salmonella* framework for poultry processing operations. One of its public health goals is to achieve a 25% reduction of *Salmonella* infections by the year 2030.

Since 2015, poultry has remained the most widely consumed animal protein in the United States. According to the National Chicken Council, the average per capita consumption of

chicken reached approximately 115.9 pounds in 2023, demonstrating its significant role in the American diet (*National Chicken Council*, 2021). However, the growing popularity of these dietary trends is not without consequence. Consumer demand for alternatively produced poultry, such as antibiotic-free, organic, and free-range options, has now surpassed that of conventionally raised chicken. While these production methods are perceived by consumers as safer due to the absence of antibiotics, pesticides, and vaccines, there is limited scientific evidence to support these assumptions (Golden & Mishra, 2020; Sofos, 2008). *Salmonella* contamination of food products can occur through both vertical and horizontal transmission pathways. Horizontally, contamination can occur most commonly through the fecal-oral route (Huis In'T Veld, 2009). Vertically, infection can occur from breeder hens to offspring via egg internal contents or the shell during laying (Huis In'T Veld, 2009). This mode of transmission highlights the potential vulnerability that alternative methods without antibiotics could potentially lead to the contamination of poultry and poultry products.

Salmonella enterica in poultry feces is considered reasonably likely to occur (RLTO) due to its ubiquity in broiler chicken houses (Chavez-Velado et al., 2024; Rai & Bai, 2017). Data from the 2021 National Antimicrobial Resistance Monitoring System (NARMS) Integrated Report Summary identified the top three serotypes among *Salmonella* isolated from retail chicken meat as Kentucky (38%), Enteritidis (23.3%), and Heidelberg (8.3%) (FDA, 2021). Other sources have also reported that *S.* Enteritidis*,* Newport and Typhimurium are the top three reported serovars causing *Salmonella* infection (CDC, 2022, 2024; Ferrari et al., 2019; Foley et al., 2011; Williams et al., 2025). Although shifts in the predominant *Salmonella* serovars have been observed over recent decades, there is limited evidence identifying consistent trends over time and across geographical regions (Williams et al., 2025). Ongoing research aims to reduce

the prevalence of *Salmonella* in poultry products, but this pathogen remains excluded from the zero-tolerance list for raw poultry due to its high likelihood of being present.

Salmonella presence in poultry has been researched by several sources as an effort to identify high-risk areas and mitigate contamination. Golden and Mishra conducted a meta-analysis showing that *Salmonella* was highest at the pre-chill stage (68.6%), followed by rehang (42.9%), and lowest at post-chill (14.3%) (Golden & Mishra, 2020). This evidence supports the Hazard Analysis and Critical Control Points (HACCP) in place to limit the growth of *Salmonella enterica* in raw poultry. Such critical control points can include monitoring of carcasses for fecal contamination, the utilization of peracetic acid as an antimicrobial during pre-chill operations, and chilling of all products (FSIS, n.d.). Another bio-mapping study identified live receiving, rehang, and pre-chill as high areas of *Salmonella* contamination across three poultry processing plants (Chavez-Velado et al., 2024). Live receiving is the point of entry for birds to the facility; rehang is the step immediately after the scalding and picking process; prechill is before the immersion chilling step, which remains the most significant critical control point in most operations due to the significant log reduction of bacterial counts from a mean concentration of 2.39 ± 0.23 log CFU/sample at live receiving to approximately 0.25 ± 0.07 log CFU/sample during the post-chill step. (Chavez-Velado et al., 2024). Discussion of this evidence suggested that by comparing the *Salmonella* prevalence of different areas located within a processing plant, strengths and weaknesses can be recognized and evaluated for potential revision.

As the global population increases, so does the pressure on the food industry to ensure a steady and sustainable food supply while minimizing waste (Quintieri et al., 2021). Traditional microbial challenge testing, which simulates environmental conditions to estimate product shelf-life, has remained the preferred method for evaluation; however, it is often criticized for being

time-consuming, labor-intensive, and economically burdensome (Baranyi & Roberts, 1994). Predictive modeling is a robust statistical tool in the food industry to estimate the fate of targeted bacteria under specific intrinsic or extrinsic conditions that may occur during processing, distribution, and storage of food products (Thomas et al., 2019). These models can be structured to consider processing interventions that can determine food safety or optimize the processing conditions. Food safety interventions include antimicrobial additions, temperature change, thermal processing, acidification, etc. In cases where unexpected deviations from product speculations arise, modeling tools can be applied to predict pathogen or bacterial growth, survival, or inactivation. These models assist in evaluating the product's condition and determining whether it presents a risk to food safety or quality. The objective of this study was to determine the minimum and maximum growth temperatures for a *Salmonella enterica* cocktail in raw ground poultry meat, develop a predictive model for the growth of *Salmonella enterica* under a full growth temperature profile (7 to 45 °C), and to validate the developed model by using a high and low temperature profile under sinusoidal temperature fluctuations.

4.3 Materials and Methods

4.3.1 Ground Chicken Meat

Poultry meat (breasts and thighs) was purchased from a local grocery store and further processed using equipment in the food processing pilot plants in the Department of Food Science and Technology at the University of Georgia. The meat was ground twice through a fine grind (1/8mm grind plate). The ground chicken was then packaged (8" x 10", Clarity 3 mil Standard Bags, Clarity Brand) and vacuum sealed with approximately 120g each prior to being frozen. The frozen bags were packaged in perishable shipping kits and sent off for electron beam

irradiation at the National Center for Electron Beam Research at Texas A&M University in College Station, Texas. The bags were sterilized with irradiation technology at 3.5kGy for total degradation of all bacteria. The meat was then stored at -20°C prior to being thawed at 4 °C 12 h prior to use. Following irradiation, various random samples were used to measure the average pH, 6.06 ± 0.06. The fat concentration was measured as 3.17± 0.17 by the ANKOM^{XT15} Extraction System (ANKOM Technology, Macedon, NY) at the University of Georgia Department of Animal and Dairy Science.

4.3.2 Bacterial Strains and Inoculation

The bacterial strains used in this study include *Salmonella enterica* serovars Hadar (S-24, turkey meat isolate), Heidelberg (S-27; environmental isolate), Montevideo (S-26; beef isolate), and Thompson (S-20; chicken meat isolate) from FSIS, and Enteritidis Phage Type 4 (S-42; clinical isolate) from the CDC. The strains were cultured from the glycerol stocks in the -80 °C storage. A 10 uL loop of the stock was used to inoculate a sterile test tube with 10mL of Tryptic Soy Broth (Becton, Dickison and Company; Sparks MD) and grown at 37 °C for 16 h to obtain an inoculum population of ~ 8.5-9.0 log CFU/mL. The inoculum was further diluted in 0.1% Buffered Peptone Water (BPW; Becton, Dickison and Company; Sparks, MD) to target a starting population of 2.5-3.0 log CFU/g in the meat pouches across all replicates and temperature experiments. Each culture tube was vortexed and 1mL of *S. enterica* strain was distributed into a sterile test tube to obtain 5mL *S. enterica* cocktail that was then serially diluted in 9mL of 0.1% Buffered Peptone Water (BPW; Becton, Dickison and Company; Sparks MD). The biological culture cocktail was used to inoculate two separate thawed bags of 120 g of irradiated ground

poultry meat. The inoculum volume was determined by the weight of the meat with approximately 10 uL of inoculum per gram of meat, to achieve a targeted initial population of 2.5-3.0 log CFU/g in the meat pouches. The ground poultry pouches were then heat-sealed and homogenized by hand massaging for two min. Small pouches (3.3”x2.5”; Clarity 3 mil Standard Bags, Clarity Brand) of meat were made with 5 ± 0.10g of inoculated meat and heat sealed. The pouches were then submerged in a circulating water bath (PolyScience, Cole-Parmer Instrument Company, Niles, IL) for the predicted duration time, for isothermal water bath temperatures at 25 °C and lower, different water bath incubation methods were used because of the incapability of the thermal water baths to maintain a temperature lower than ambient temperature. A heating water bath was placed in a walk-in cooler set to 4 °C. This allowed the circulating water bath to maintain constant temperatures from 25-8 °C. Due to limited equipment availability, temperature incubation was achieved using alternative methods. For the 7 °C temperature experiments, a water-filled container was placed inside a refrigerator to maintain consistent and controlled environmental temperatures, and temperature was monitored with a data logger (Fischerbrand TraceableLIVE- Thermo Fischer Scientific Inc.).

4.3.3 Sampling

At the planned fixed time point for sampling, a pair of replicate inoculated meat pouches was removed from the water bath, and the meat from the pouches was aseptically transferred into filtered Whirl-Pak bags (Nasco International, Madison, WI) with 20mL of 0.1% BPW (Becton, Dickison and Company; Sparks MD). The pouches were then hand massaged for two min, and a series of tenfold serial dilutions were prepared based on preliminary predictions (Juneja et al.,

2007; Combase) The selected dilutions were then plated in duplicates on Aerobic Count Petrifilms (3M, St. Paul, MN) in 1.0 mL increments and incubated for 24 h at 37 °C to enumerate surviving *Salmonella* populations. To assure no background microflora were present throughout the study random samples were plated on xylose, lysine, and sodium deoxycholate agar (XLD; Becton, Dickison and Company; Sparks MD) to visually inspect the black color of the colonies, not for enumeration.

4.3.4 Predictive Modeling

Primary Model

A primary model describes the baseline distribution of how a microbial population grows, survives, or dies under isothermal, or constant conditions over time. One of the most widely used primary models in predictive microbiology is the Baranyi model. It is important to select the most appropriate type of primary model for a dataset based on the intended application and highest accuracy. The Baranyi model is particularly effective for growth prediction because it characterizes the complete microbial growth curve, including the lag, exponential, and stationary phases along the entire sigmoidal curve (Baranyi & Roberts, 1994; Thomas et al., 2019). This model captures the time taken by bacterial cells to adjust to their environment, or the physiological adaptation referred to as the lag phase (Thomas et al., 2019). Once isothermal data was collected across different temperature conditions, the log-transformed bacterial counts (log CFU/mL) at given time points were fitted to the Baranyi primary modeling equation using the USDA Integrated Pathogen Modeling Program 2013 (IPMP) software. The equation for the fitted model is as follows:

Baranyi Model equation:

$$y(t) = y_0 + \mu_{max}F(t) - \ln\left(1 + \left(\frac{e^{\mu_{max}F(t)} - 1}{e^{(y_{max} - y_0)}}\right)\right)$$

where $F(t) = t + \frac{1}{v}\ln\left(e^{-vt} + e^{h0} - e^{(vt-h0)}\right)$. The parameters of the equation are defined as: $y(t)$is the cell population of *S. enterica* (log CFU/g) at time t, $y_0$is the initial cell population (log CFU/g), $y_{max}$is the maximum cell population reached (log CFU/g), μ_{max} is the specific growth rate (hours), h_0 describes the prior physical state of the cells. The lag phase duration was calculated using the formula: $\lambda = \frac{h_0}{\mu_{max}}$ where λ is the parameter for lag phase duration (h) (Zwietering et al., 1991).

Secondary Modeling

A secondary model is used to integrate the impact of the environmental parameter used in the primary model. The Ratkowsky square root model frequently provides an accurate fit during bacterial growth studies by interpreting the biological parameters The parameters given from the primary modeling output were used to develop a secondary model to demonstrate the relationship of temperature and specific growth rate (μ_{max}). The values from each replicate were fitted into with the secondary Ratkowsky Square Root model (Ratkowsky et al., 1981) The equation is as follows:

$$\mu_{max} = a(T - T_{min})^2$$

where the a parameter is the regression coefficient, T is the temperature (°C), and T_{min} is the minimum temperature (°C). This was also preformed using the USDA Integrated Pathogen Modeling Program 2013 (IPMP) software (Huang, 2013).

Tertiary Model

Tertiary models are user-friendly software tools designed to accurately predict bacterial behavior under specific conditions. These models are built upon validated primary and secondary models under dynamic conditions. One of the most widely used software's was developed by the USDA-Agricultural Research Service (USDA-ARS) known as the Integrated Pathogen Modeling Program (IPMP) (Huang, 2013). IPMP enables users to simulate bacterial behavior during growth, survival, or inactivation in response to various environmental changes such as temperature, pH, water activity (Aw), etc. Despite its utility, a major limitation of IPMP is that it is restricted to the access of only raw data, limiting its flexibility. Another common USDA-ARS software applied is ComBase (Barayni & Tamplin, 2004), which includes access to an extensive database with over 65,000 records detailing the behavior of pathogens and spoilage organisms across a wide range of food matrices.

4.4 Statistical Analysis

The statistical evaluation of the developed primary models during the present study involved comparing goodness-of-fit metrics, including Root Mean Squared Error (RMSE), Sum of Squared Errors (SSE), Mean of Squared Error (MSE), and Residual Standard Deviation. These metrics were generated as part of the output following the fitting of the Baranyi model to the experimental data using the USDA-ARS Integrated Pathogen Modeling Program (IPMP). The corresponding equations for each metric are presented in Table 4.1.

For the secondary model, analysis focused on RMSE and identifying trends observed during bacterial growth such as maximum specific growth rate (μ_{max}) and lag phase duration (LPD). Evaluation of the tertiary was based on accuracy and bias factors, calculated using MATLAB software (Baranyi et al., 1999).

4.4.1 Validation of Models

The tertiary predictive growth model developed was validated using two dynamic sinusoidal temperature profiles under controlled intrinsic conditions: a low temperature profile (7-25°C, 12-hour cycles), and a high temperature profile (20-45°C, 8-hour cycles). As previously mentioned, inoculated samples were placed in a circulating programmable water bath to simulate temperature fluctuations at fixed intervals. Sampling was conducted at planned time intervals, and the growth of *Salmonella enterica* was recorded until cells reached the stationary phase.

4.5 Results and Discussion

4.5.1 Temperature Threshold

The *Salmonella enterica* strains tested in the present study exhibited growth in raw ground poultry meat under constant isothermal condition from temperatures 7 to 45°C. While determining the minimum and maximum temperatures during preliminary studies, it was found that this specific cocktail of *S. enterica* did not exhibit growth at temperatures below 7°C and exhibited limited growth by only increasing approximately 1.4 log CFU/g between 14 and 20 days before decreasing as shown in Figure 4.1. The limit of detection (LOD) for *Salmonella* was

0.35 log CFU/g when plated on APC Petri films. For the upper threshold limit, *S. enterica* growth was not detected at temperatures higher than 45°C (LOD = 0.35). According to Matches & Liston (1968), this is normal behavior for most *S. enterica* strains. and the temperature threshold for *Salmonella* can vary based on species and nutrient medium (Matches & Liston, 1968).

4.5.2 Primary Modeling

This study measured the growth of *Salmonella enterica* at nine different temperatures: 7, 10, 15, 20, 25, 30, 35, 40, and 45°C. The bacterial growth from each of the isothermal experiments was measured, converted to log CFU/g, and fitted with the Baranyi equation using the USDA-ARS Integrated Pathogen Modeling Program (Figure 4.1). The growth parameters: maximum specific growth rate (μ_{max}), lag phase duration (LPD, λ), initial cell concentration (y0), and maximum cell population (y_{max}) were recorded (Table 4.2). The parameter h0 is characterized as the 'adaptation work' required of a bacteria to adapt existing conditions (Juneja et al., 2007; Thomas et al., 2019; Tsaloumi et al., 2022). It is assumed in the Baranyi equation that the value of h0 is constant for a given bacterial species in a specific food matrix (Baranyi & Roberts, 1994; Juneja et al., 2007; Thomas et al., 2019). In this study, there was a slight correlation between a decreasing h0 value and increasing temperature; however, because of the decision to include the 7°C temperature data with minimal growth, this created a slight deviation in the trend. Therefore, the arithmetic mean of h0 (1.35) was used for further modeling development. The primary models were refitted with the new h0 value, and the other parameters were recalculated.

The initial cell concentration for the inoculated meat pouches ranged from 2.79-3.24 log CFU/g across all temperature experiments compared to the targeted inoculation of 2.5-3.0 log CFU/g (Table 4.2). The maximum population did not exceed 9.18 log CFU/g during any of the isothermal studies, and there was no observed correlation between initial inoculation level and maximum population density.

Although goodness-of-fit metrics are recommended for relative rather than absolute evaluation, they can still offer valuable insight into a model's performance. The corresponding equations for each metric of note are presented in Table 4.1. In this study, the Baranyi model was fitted to primary isothermal growth data, resulting in low Root Mean Squared Error (RMSE) values ranging from 0.065 to 0.303 (Table 4.3), suggesting a strong similarity between observed and predicted values. In general, lower RMSE, SSE, and MSE values—those approaching zero—indicate a better fit to the data. The Sum of Squared Errors (SSE) was also provided as a comparative metric, which is typically useful when evaluating multiple models. However, because only the Baranyi model was applied in this analysis, SSE values are of limited relevance for assessing model performance in this context, but they can provide valuable insight for comparing future research with additional model equations (Table 4.3). Additionally, Mean Squared Error (MSE) values, ranging from 0.0045 to 0.121, were generated by the modeling software. Unlike RMSE, which retains the same units as the observed data, MSE is expressed in squared units. While MSE is commonly used by model developers to assess optimization and performance across various model types, it holds less interpretive value in the current single-model framework.

4.5.3 Secondary Modeling

The observed maximum specific growth rate (μ_{max}) exhibited a positive correlation with temperature increasing steadily up to the optimal growth temperature of 40°C, followed by a slight decline at 45°C (Figure 4.2). When the data were fitted to the Ratkowsky model, the resulting Root Mean Squared Error (RMSE) of 0.083 supports the idea that the observed values closely aligned with the predicted outcome, indicating a high level of model accuracy. The lag phase duration (LPD)-which reflects the time required for bacterial cells to physiologically adapt to new environmental conditions- showed a negatively exponential relationship with temperature reaching its shortest duration at its optimal temperature of 40 °C, after which it began to rise again (Figure 4.3). This trend aligns with findings from previous studies. According to Baranyi et al. (1995), LPD is temperature-dependent, and exposure to temperatures near the lower threshold may trigger cold shock, prolonging the lag phase. The results in the present study support this interpretation. The Ratkowsky reported that the mean predicted minimum temperature (T_{min}) for growth was 9.44 ± 1.56°C and the mean predicted maximum growth temperature (T_{max}) was 45.23 ±0.02°C for *S. enterica* in raw ground poultry meat. These values remain close to the observed minimum (7°C) and maximum (45°C) temperatures, and is consistent with existing literature (Juneja et al., 2007; Milkievicz et al., 2020).

4.5.4 Growth Models Under Dynamic Temperature Profiles and Validation Evaluation

Figure 4.4 presents the validated tertiary models subjected to a dynamic temperature profile with their corresponding prediction curve. For the lower temperature profile (7-25°C), the produced accuracy factor in response to the fitted prediction was 1.044, and a bias factor of

0.9771. The higher temperature profile (20-45°C) resulted in an accuracy factor of 1.034 and a bias factor of 1.012. The Accuracy factor measures how closely the predicted values align with experimental observations by quantifying the overall deviation (Baranyi et al., 1999). The Bias factor indicated the direction of deviation, whether the model tends to overpredict or underpredict bacterial behavior. For both metrics, values approaching 1.0 signify a high level of model reliability. An Accuracy Prediction Zone (APZ) analysis was performed to assess the degree of deviation between the observed and predicted bacterial counts (Figure 4.5). The predicted error was calculated by subtracting the observed values from the predicted values. The acceptable range for prediction error was defined between -1.0 log CFU/g (fail-safe) and 0.5 log CFU/g (fail-dangerous). It is acknowledged that some deviations are to be expected when modeling the bacterial behavior in a complex food system. Overprediction (fail-safe) suggests a conservative model, while underprediction (fail-dangerous) can pose as a greater risk by underestimating the bacterial growth and the potential for solicitation and consumption of contaminated food product (Juneja et al., 2018; Oscar, 2005; Ross et al., 2000; Thomas et al., 2019). However, overprediction is still not desired as it can warrant unnecessary disposal of potentially safe food, and result in substantial economic losses (Thomas et al., 2019). This analysis yielded an APZ score of 96% with only one out of 25 points from both temperature profiles falling outside of the accepted accuracy prediction zone (-1.0 to 0.5 log CFU/g) (Figure 4.6). An APZ score of >70% is preferred and considered as an acceptable score (Oscar, 2005). This indicates a high level of agreement between the observed and predicted values, thus demonstrating a strong model performance under dynamic conditions.

4.6 Conclusion

This study successfully characterized the growth behavior of *Salmonella enterica* across the entire growth kinetic temperature range using predictive modeling. The Baranyi equation accurately described isothermal growth, evidenced by a low RMSE range (0.065-0.303), and consistent initial and maximum population levels. Utilizing the arithmetic mean h0 value (1.35) yielded consistent model performance across all temperatures. The secondary model fitted with the Baranyi equation demonstrated a strong correlation between temperature and both maximum specific growth rate (μ_{max}) and lag phase duration (LPD, λ). The tertiary model validation profiles confirmed the model's reliability and predictive strength based on the accuracy and bias factors with values near 1.0, and a high APZ score of 96%. Overall, the data from model can serve as an effective predictive tool for users to simulate the growth of *Salmonella enterica* in raw ground poultry meat subjected to abusive temperature deviation.

4.7 References

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LIST OF FIGURES

Figure 4.1

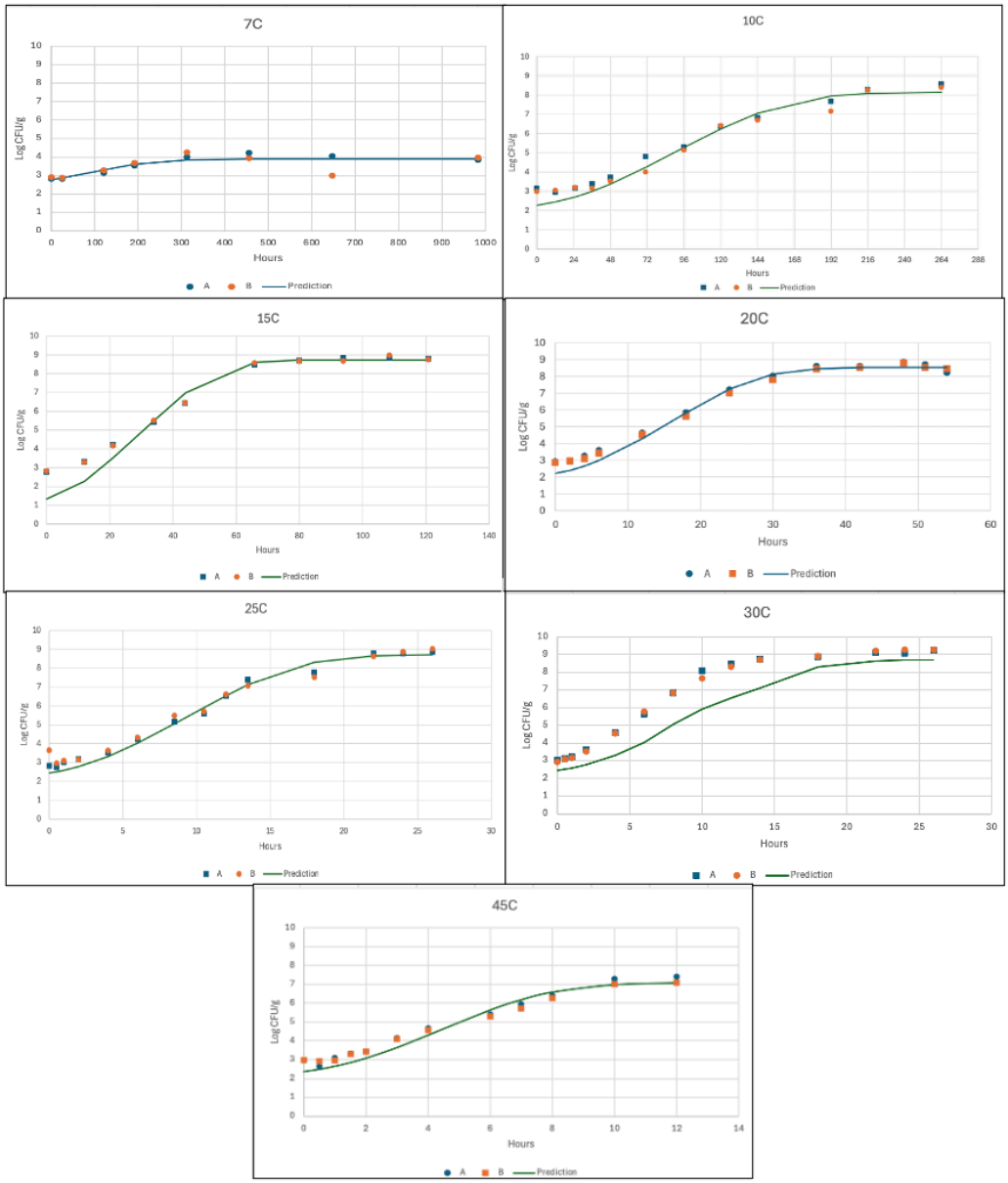

Figure 4.2

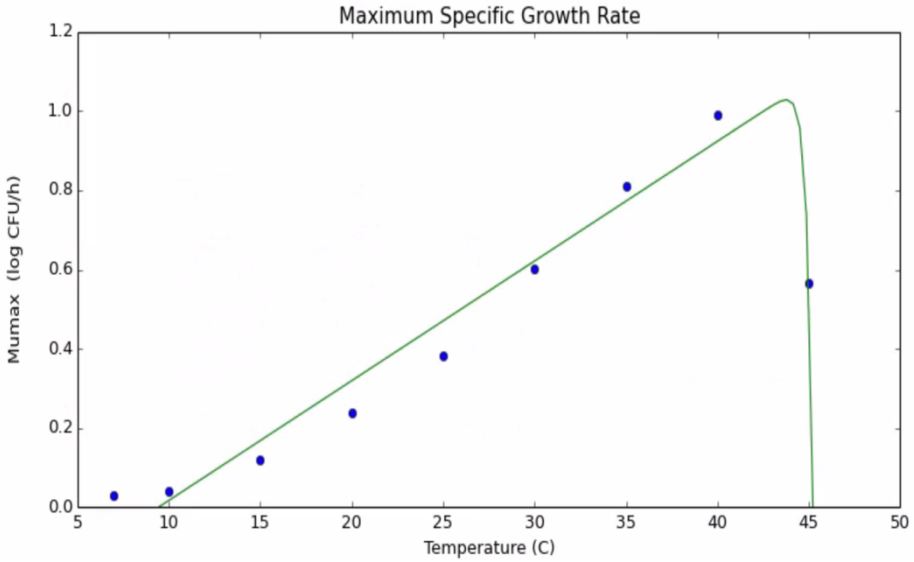
Maximum Specific Growth Rate
Mumax (log CFU/h)
Temperature (C)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
5
10
15
20
25
30
35
40
45
50

Figure 4.3

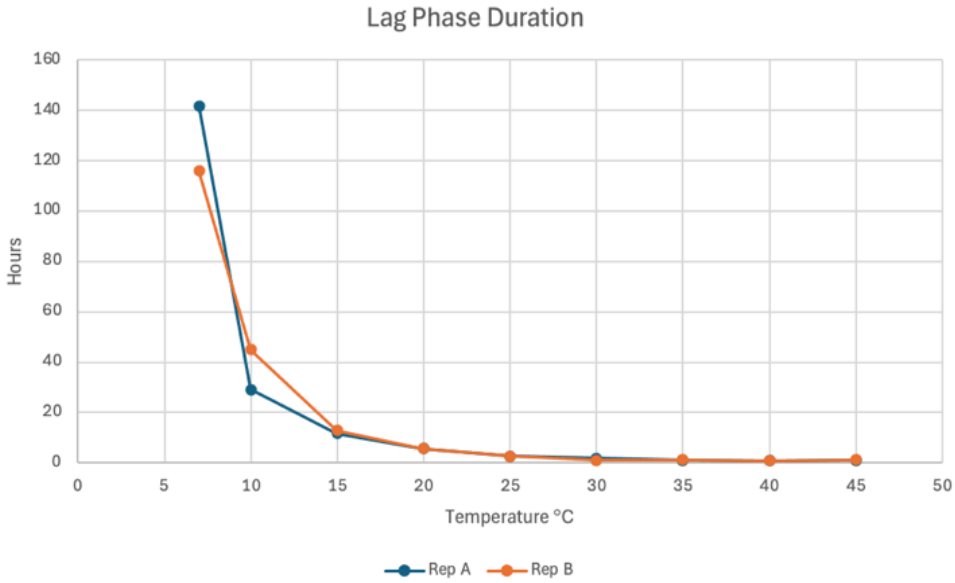
Lag Phase Duration
160
140
120
100
80
60
40
20
0
Hours
0
5
10
15
20
25
30
35
40
45
50
Temperature °C
Rep A
Rep B

Figure 4.4

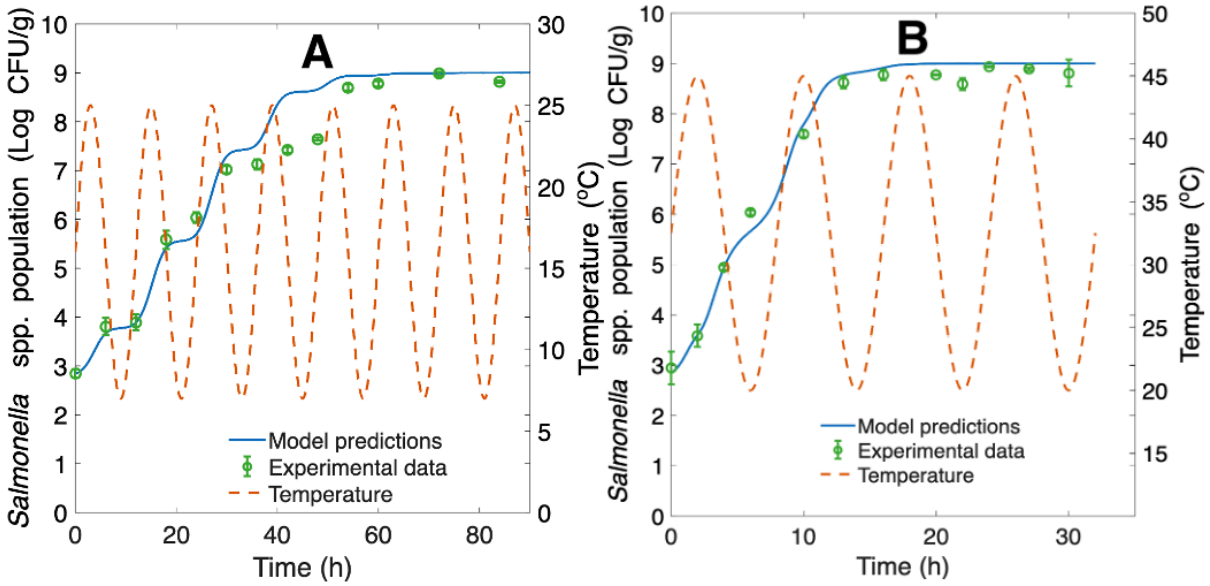
A
B
Salmonella spp. population (Log CFU/g)
Temperature (°C)
Time (h)
Model predictions
Experimental data
Temperature

Figure 4.5:

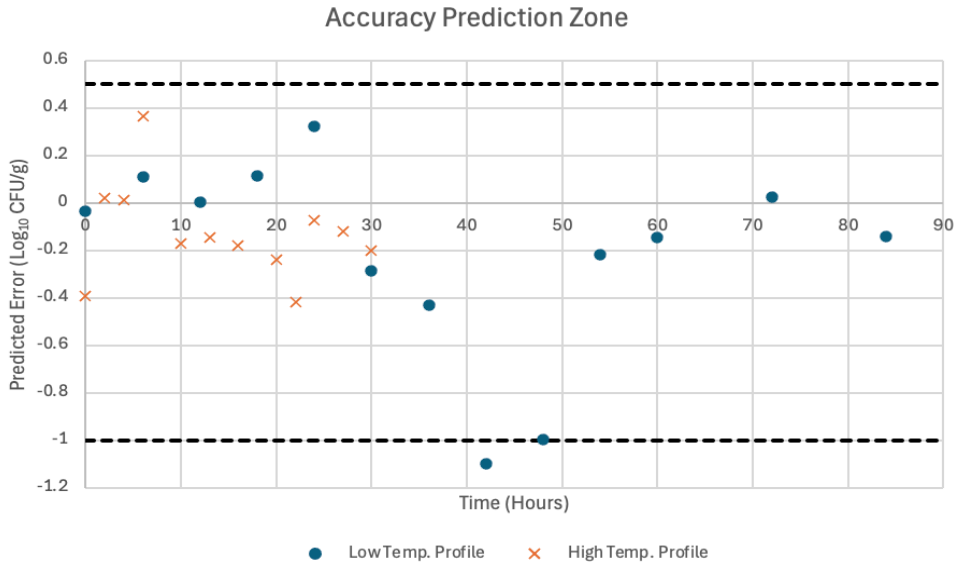
Accuracy Prediction Zone
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
0
10
20
30
40
50
60
70
80
90
Predicted Error (Log10 CFU/g)
Time (Hours)
Low Temp. Profile
High Temp. Profile

Table 4.1: Goodness of fit metrics and the associated formulas.

Metric	Name	Formula
SSE	Sum of Squared Errors	$\sum_{i=1}^{n}(y_i - \hat{y}_i)^2$
MSE	Mean Squared Error	$\frac{1}{n}\sum_{i=1}^{n}(y_i - \hat{y}_i)^2$
RMSE	Root Mean Squared Error	$\sqrt{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}$
RESIDUAL SD	Residual Standard Deviation	$\sqrt{\frac{SSE}{n-p}}$

Table 4.2: Specific growth rate(μ_{max}), maximum population density (y_{max}), lag-phase duration (λ), and initial bacterial concentration (y0) of *S. enterica* in raw ground poultry meat at different temperatures

Temp (ºC)	**μ_{max} (log CFU/h)**	**λ (h)**	**y0 (log CFU/g)**	**y_{max} (log CFU/g)**
7	0.03	128.81	2.87	3.91
10	0.04	36.92	3.06	8.33
15	0.12	12.26	2.79	8.83
20	0.24	5.62	2.88	8.61
25	0.38	2.64	3.24	8.93
30	0.60	1.45	2.98	9.13
35	0.81	1.05	2.98	9.18
40	0.99	0.86	2.96	8.87
45	0.57	1.12	2.97	7.54

Table 4.3: Primary modeling Goodness of Fit Metrics extracted from the USDA Pathogen Modeling Program Software for *S. enterica*

Temp (ºC).	RMSE	SSE	MSE	SD
7	0.30	0.48	0.12	0.21
10	0.27	0.61	0.08	0.22
15	0.11	0.08	0.01	0.09
20	0.14	0.18	0.02	0.11
25	0.26	0.71	0.07	0.22
30	0.12	0.15	0.02	0.10
35	0.07	0.03	0.01	0.05
40	0.09	0.06	0.01	0.07
45	0.16	0.21	0.03	0.13

Chapter 5

OVERALL CONCLUSION AND FUTURE RESEARCH

The risks associated with bacterial contamination of both *Pseudomonas putida* and *Salmonella enterica* in raw ground poultry meat are substantial from an economic and public health perspective, thus amplifying the need for more effective approaches to aid in the mitigation of the impact from the presence of these bacteria. The development and validation of the predictive growth models for *P. putida* and *S. enterica* provides a valuable tool for food industry application in both quality and safety risk assessments. These studies demonstrated the distinct growth patterns associated with each bacterium under their specific growth temperature range and their response to changing temperature conditions. The shifts in environment were captured with both the Baranyi and Ratkowsky models, as they considered the stages of bacterial growth and measured the bacterial responses. The integrated data from each of the primary and secondary models allowed for a representative tertiary model and dynamic validation that closely mimicked real-world product temperatures during processing, transport, and storage.

Model performance during each stage of development was evaluated, and it confirmed the reliability and consistency of the observed data as presented in the RMSE metric values for the primary and secondary models, and the Accuracy and Bias factors for the tertiary model. The main findings of this study included that the maximum specific growth rate and lag phase

duration are affected by temperature. Both studies indicated that lag phase duration exponentially decreases with increasing temperature up to the optimal growth temperature (30 °C for *P. putida* and 40 °C for *S. enterica*), and maximum specific growth rate ($\mu_{max)}$ increases with temperature up to the optimal growth temperature.

The *P. putida* model serves as an effective tool for shelf-life determination and spoilage risk assessment that holds promise to minimize the amount of unnecessary food waste due to microbial spoilage and contribute to increased long-term sustainability practices. Similarly, the *S. enterica* model can offer a more advanced and expedited approach for risk assessment in suspected pathogenic contamination of poultry products. This can promote the evaluation of HACCP operations and serve as a diagnostic tool during the auditing of antimicrobial interventions.

Future studies should explore additional environmental variables that influence the bacterial growth kinetics. For *P. putida*, studies investigating the impact of modified atmosphere packaging (MAP) with varying oxygen concentrations are recommended to identify an approximate minimum concentration of oxygen required for *P. putida* growth. Future research for both *Salmonella* and *Pseudomonas* predictive modeling should also consider the role of competing background microbiota during the survival of the bacteria in raw ground poultry meat. Additionally, the influence of varying fat concentrations on bacterial persistence and lag phase duration warrants investigation to enhance model accuracy under more diverse food matrix conditions.