DEVELOPMENT AND VALIDATION OF DYNAMIC PREDICTIVE MODEL FOR THE GROWTH OF BACILLUS CEREUS SPORES IN READY-TO-EAT (RTE) TURKEY BREAST, ROAST BEEF AND HAM

by

SUJITHA BHUMANAPALLI

(Under the Direction of Harshavardhan Thippareddi)

ABSTRACT

Dynamic predictive models for the *B. cereus* growth in RTE turkey breast, roast beef, and ham were developed and validated. Freshly prepared meat samples were inoculated with *B. cereus* spores, heat-treated and stored at various isothermal temperatures (10 - 55ºC). *B. cereus* growth data obtained at each temperature were fitted using the primary Baranyi model. The growth rates obtained from the primary model were fitted using the secondary Ratkowsky model as a function of temperature. The models showed high accuracy (R^2 values: 0.9873 and 0.9435, respectively). Tertiary models for non-isothermal profiles were developed and validated using the acceptable zone analysis (APZ) method. Over 93% (average) of observations fell within the APZ limits (-1.0 < predictive errors < 0.5 log CFU/g). The developed models will help food industry and regulatory agencies to predict *B. cereus* growth under extended storage and temperature abuse scenarios to ensure microbiological safety of cooked meat products.

INDEX WORDS: *Bacillus cereus*, beef, ham, predictive microbiology, turkey

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SUJITHA BHUMANAPALLI

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SUJITHA BHUMANAPALLI

Major Professor: Harshavardhan Thippareddi
Committee members: Abhinav Mishra
Alexander Stelzleni

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
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DEDICATION

I would like to dedicate my work to my loving and supportive husband (Venkata Sesha Reddy Choppa), parents (Rama Devi & Vishnu Pratap Reddy), sister's family (Vedavathi & Prasad) and sisters-in-law's families (Dr. Nirmala & Dr. Vijay; Pallavi & Aravind).

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CHAPTER 1

INTRODUCTION

Foodborne illnesses exert a substantial global impact annually, affecting over 550 million individuals and leading to an estimated 230,000 fatalities. (Diplock, 2023). Approximately one in six people in the United States suffer from a foodborne illness, resulting in approximately 48 million illnesses, 128,000 hospitalizations, and 3,000 fatalities per year (CDC 2016). As documented by the National Outbreak Reporting System (NORS), *Bacillus cereus* has been linked to 754 outbreaks resulting in 10,568 illnesses, 144 hospitalizations, and 5 deaths between 2009 and 2020. As a spore-forming Gram-positive bacteria, *B. cereus* spores often survive thermal treatment and start germination and outgrowth in cooked foods under temperature-abusive conditions. A Rhode Island nursing facility had a *B. cereus* gastroenteritis epidemic after the residents and employees consumed inadequately preserved beef stew. The symptoms, including diarrhea and pain, lasted 16 hours, but no deaths were reported. Moreover, *B. cereus* was detected in stool samples from 10 out of 23 unwell patients and 1 out of 21 healthy controls. All *B. cereus* isolates were H.26 and generated diarrheal enterotoxin in rabbits. All isolates showed identical plasmid profiles that differed from *B. cereus* strains from earlier outbreaks (DeBuono et al., 1988). Similarly, 22% of 643 respondents to postal questionnaires reported diarrhea after a university field trip day. Participants who consumed barbecued pork that was left unrefrigerated for 18 hours were found to be five times more associated with developing foodborne illness compared to non-pork eaters, with an illness rate of 26% among the pork consumers, in contrast to a rate of 5%

among non-pork eaters. *Bacillus cereus* isolated from the leftover pork produced a diarrheal toxin below 5 log CFU/g. 34% of people experienced illness, beginning outside the normal 6 to 24-hour *B. cereus*-mediated diarrhea incubation period, and 23% developed fever. These data implied that reported foodborne illness induced by *B. cereus* is only the tip of the iceberg for total cases (Luby et al., 1993).

Despite the advances in food technology and processing, certain food commodities are associated with microbiological hazards, particularly when temperature abuse occurs during transportation and storage. Predictive microbiology is a scientific-based tool covering an integrated approach that improves food safety. Several predictive models on *B. cereus* have been reported on cereal or beans product to minimize the risk (Albaridi, 2022; Juneja et al., 2017), however, there is a lack of information on the predictive models of *B. cereus* on meat and poultry products. To alleviate the *B. cereus* outbreak risk, an effective predictive growth model is urgent to examine the growth of *B. cereus* within a controlled environment (isothermal) to construct the predictive mathematical models of *B. cereus*. The primary models are to describe the growth and behavior of *B. cereus* with time at isothermal conditions. While the secondary model aims to determine the relationship between model parameters (obtained from primary models) and the environmental conditions. Subsequently, models were validated by comparing the predicted values with actual observations. The overall goal of this study was to develop dynamic predictive models for the growth of *B. cereus* in cooked ready-to-eat poultry and meat products with the following specific objectives:

1. To investigate the growth of *B. cereus* across various isothermal conditions ranging from 10 and 55°C with a 5°C increment and to develop a dynamic predictive model for its growth in RTE turkey breast, roast beef, and ham.
2. To validate the developed growth model using two sinusoidal temperature

profiles and compare the results using acceptable prediction zone (APZ) analysis.

CHAPTER 2

LITERATURE REVIEW

BACILLUS CEREUS

Bacillus cereus and Epidemiology

Bacillus cereus is gram-positive, motile (flagellated), and spore forming microorganism that belongs to the *Bacillus* genus. Other species within this genus include *B. anthracis, B. mycoides, B. thuringiensis, B. pseudomycoides, and B. weihenstephanensis*. The genomic sequences revealed almost similar 16s rRNA sequences among *B. anthracis, B. cereus, and B. thuringiensis* (Montville & Matthews, 2005; Rajkowski & Bennett, 2003). Among these, *B. cereus* is omnipresent in different environments and follows a saprophytic life cycle, undergoing germination, growth, and sporulation (Vilain et al., 2006). Spores are more resistant to environmental stressors due to their metabolic dormancy and physical resilience but germinate upon contact with organic matter or an animal host or arthropods, followed by sporulation of vegetative cells, maintaining the life cycle (Jenson & Moir, 2003; Turnbull & Kramer, 1985). Besides the saprophytic characteristics the endospores possess, the adhesive nature makes them prevalent in the food production systems and colonizes the intestine causing peril (Turnbull & Kramer, 1985). These cellular characteristics and ubiquitous nature gained the focus of researchers concerning their growth patterns in varying environmental conditions and developed mathematical models useful for food safety regulatory agencies and food production units (Ehling-Schulz et al., 2006).

Due to its extensive range of phenotypic characteristics, *B. cereus* can infiltrate food production and processing chains at many points, posing numerous issues to the food industry. In addition to

its potential to cause food spoiling, it poses a serious health issue to consumers. *B. cereus* is associated with two types of foodborne illnesses, emetic and diarrheal. Both illnesses are usually mild and resolve on their own, but in some cases, they can be severe and even fatal (Stenfors Arnesen et al., 2008). Precisely, non-hemolytic enterotoxin complex (NHE) and the hemolytic enterotoxin complex (HBL), as well as a variation of the single cytotoxin K, have been connected to the diarrheal form of the disease, cereulide being identified as the agent responsible for the emetic form (Ehling-Schulz & Messelhäusser, 2012). Two enterotoxin complexes and one single enterotoxin protein have been suggested to be associated with this type of food poisoning, but only the role of two enterotoxin complexes (HBL and NHE) has been confirmed (Agata et al., 1996; Lund & Granum, 1996). These enterotoxin complexes can be detected by commercial immunoassays (Kotiranta et al., 2000). The emetic type of food poisoning is caused by a dodecadepsipeptide cereulide, and the symptoms include nausea, vomiting, and occasionally diarrhea, which appear within one to five h after ingestion and lasts less than 24 h (Lund & Granum, 1996; Shinagawa et al., 1995).

Bacillus cereus is responsible for both food intoxication and toxico-infection by producing enterotoxin complexes (Stenfors Arnesen et al., 2008). The emetic nature pertains to cerulide, heat stable depsipeptide which is pre-formed in the various foods associated with hospitalizations and intoxications with fatality are being reported in higher numbers (Carlin et al., 2006; Ehling-Schulz et al., 2010; Stenfors Arnesen et al., 2008). Although there is limited research on the exact number of cells required for *B. cereus* to initiate cereulide production in food, 5 log CFU/g is estimated as the threshold for intoxication (Jovanovic et al., 2021). Additionally, diarrheal form resembles *Clostridium perfringens* infection characterized by abdominal pain along with watery diarrhea. The ingestion of *B. cereus* vegetative cells and spores could induce toxic infection. Food products

containing more than 3 log CFU/g of *B. cereus* are classified as unsafe for consumption according to EFSA guideline (EFSA 2016).

Growth and Resilience Characteristics

Different strains of *B. cereus* could exhibit diverse growth and survival characteristics. For instance, mesophilic or psychrotrophic strains are defined based on their ability to grow under specific temperature conditions. To be precise, mesophilic strains thrive at 37°C but show no growth below 10°C, while psychrotrophic strains grow well at refrigeration temperatures but poorly at 37°C (Wijnands, 2008). Moreover, strains associated with emetic toxin production are predominantly mesophilic in nature (Pielaat et al., 2005; Wijnands, 2008).

B. cereus could tolerate a maximum salt concentration of 7.5% (Satapute et al., 2012). Besides, it prefers aerobic conditions for optimal growth but could survive and grow under anaerobic conditions (Rajkowski & Bennett, 2003). Interestingly, cells grown aerobically are less resistant to heat and acid compared to those grown anaerobically or micro-aerobically. Mesophilic strains demonstrate greater acid resistance than psychrotrophic strains (Mols & Abee, 2011).

B. cereus spores exhibit considerable strain variability to heat. Some heat-resistant strains have D-values (the time required to reduce the initial concentration of cells or spores by 1 log) that are 15 to 20 times higher than those of heat-sensitive strains (Augustin, 2011; Wijnands, 2008). For example, the D-value at 85°C ranges from 33.8 to 106 minutes in the phosphate buffer, while in distilled water and milk, it is ranging from 1.5 to 36.2 minutes and from 1.8 to 19.1 minutes, respectively (Foods, 1996). A higher heat resistance is found in high-fat and oily foods, such as the D-value at 121°C is 30 min in soybean oil. Spores demonstrate greater resistance to dry heat than moist heat, and their heat resistance is typically higher in foods with a lower water activity (Cho & Chung, 2020). Additionally, spores are more radiation-resistant than vegetative cells

(Jenson & Moir, 2003).

Under typical conditions like gradual cooling after improper cooking or improper cooling after cooking, *B. cereus* can produce spores quickly. (Kotiranta et al., 2000; Leive & Schlessinger, 1984). Thermophilic and psychrophilic members of *B. cereus* can survive and grow in extreme temperatures as high as 75ºC or as low as 3ºC by forming spores which germinate under optimal conditions (Drobniewski, 1993). These spores play a crucial role in the pathogenesis of *B. cereus* by attaching to human epithelial cells (Andersson et al., 1996). Moreover, the ability of spores to germinate and initiate the growth of sporangia is heavily reliant on the presence of suitable environmental conditions as mentioned earlier leading to gastrointestinal and other systemic infections (Beecher et al., 1995; Cliver & Cochrane, 1986; Kotiranta et al., 2000; Kramer & Gilbert, 1989).

Bacillus cereus Spore Structure and Formation

Bacillus species produce endospores that can resist thermal, radiation, and chemical treatments. The spores formed by *B. cereus* has an external layer called exosporium composed of a number of different collagen-like glycoproteins (ExsJ and BclA) that functioned a major role in the attachment to surfaces of the spores (Ball et al., 2008; Stewart, 2015). The exosporium of *B. cereus* is composed of 43–52% protein, 15–18% lipid, and 23% carbohydrates of dry weight. ExsJ was identified as a major glycoprotein in the *B. cereus* exosporium due to its larger N-terminus than BclA (Todd et al., 2003). BclA is a trimerized glycoprotein that is attached to the basal layer and formed the hair like surface of spores. BclA and ExsJ together set up the exosporium and are responsible for the spore adhesion and virulence (Maes et al., 2016).

The sporulation is linked to unfavorable environments; therefore, the vegetative cells require a way to sense the external stressor signals. The control of spore formation is reported by a

phosphorelay system (de Vries et al., 2004). Activation of the sporulation process occurs sequentially through the intercompartmental signals associated with sigma specific transcription factors, which is not conserved in *Clostridium* spp (Fimlaid & Shen, 2015). The sporulation of a vegetative *B. cereus* cell has been extensively described (Collado et al., 2006). It begins with the forming an asymmetrically positioned septum, dividing the cell into a smaller and larger parts through the chromatin's asymmetrical division. Subsequently, the smaller part, known as the fore spore, is engulfed by the mother cell, resulting in the formation of a dense peptidoglycan layer, referred to as the cortex, around the chromatin network. In the final stage, the mature spore is released from the mother cell through lysis, allowing the dormant spore to be released into the environment. These resulting *B. cereus* spores possess an enhanced capacity to endure harsh conditions, including pH levels ranging from 1 to 5.2, elevated temperatures such as 2.0 minutes at 95ºC or 32.1 minutes at 85 ºC, antimicrobial agents like ampicillin, cephalothin, oxacillin, as well as exposure to UV or gamma radiation (Soni et al., 2016). Once the spore is developed, it can remain in a dormant state for extended periods, spanning many years. Alternatively, when it encounters favorable environmental conditions, it can initiate the process of germination, leading to the formation of a vegetative cell (Cohn, 1875; Koch, 1877).

Spore Germination

Spore germination is the process by which dormant, dehydrated spores are transformed into vegetative cells capable of growth and reproduction. During germination of Bacillus spp., the first step entails the liberation of dipicolinic acid (DPA), succeeded by the rehydration of the core as water replaces DPA. The second step involves the breakdown of the peptidoglycan layer or cortex lysis, followed by core rehydration and expansion of the germ cell wall, thus completing the initial phase of germination. As a result of rehydration, enzyme activity is reinstated, leading to

outgrowth, which encompasses the synthesis of macromolecules (Setlow, 2003; Collado et al., 2006). The germination process of *Bacillus cereus* spores is subject to multiple variables including ambient temperature, the existence of germinants, and, under specific circumstances, heat activation. Heat activation is characterized as a sublethal thermal exposure, typically within a range of 65 to 80°C, which has been demonstrated to enhance the spore germination (Fernández et al., 2001; Luu et al., 2015). Germination is initiated when nutrients like amino acids (particularly L-alanine), glucose, and lysozyme, collectively known as germinants, bind to GerA and GerB receptors in the spore coat's inner membrane (Setlow, 2003). It is important to mention that certain *Bacillus cereus* spores possess a limited number of germinant receptors on their inner membrane, making them unresponsive to the presence of amino acids or glucose for germination induction (Ghosh and Setlow 2010). These spores, known as super-dormant, can only germinate through heat treatment, posing a risk with improperly reheated food (Setlow, 2006; Zhang et al., 2013; Zhang et al., 2010; Y. Zhang et al., 2011).

***B. cereus* in Foods**

Bacillus has been isolated in numerous global locations within a diverse range of food products such as fresh vegetables, rice, pasta, eggs, milk and dairy products, and meat products (Shi and Zhu, 2009). A list of incidences of *B. cereus* contamination in food products is listed in Table 1. Numerous factors contribute to the occurrence of *Bacillus* spores across various food industries. Here we listed some examples of common food products including fresh vegetables, cereals, eggs, milk and dairy, meat, and poultry products.

Fresh vegetables can be contaminated with enterotoxigenic *Bacillus cereus* with an estimated high prevalence of 36.94% due to the contact of soil (Rahnama et al., 2023). In most cases, consumers ingest vegetables in their natural state or after only minor processing, neither of which

is guaranteed to eradicate all potentially hazardous bacteria, such as *B. cereus* (Mogren et al., 2018). In a study that examined 102 samples of fresh vegetables, 47% were found to be contaminated with *B. cereus*, and 6.8% had a high level of contamination (>3 log CFU/g). *B. cereus* isolates from these vegetables were found to possess multiple virulence genes and some exhibited resistance to certain antibiotics. Raw vegetables containing *B. cereus* and its toxins pose potential health risks if consumed raw or undercooked (Park et al., 2018). Another study found that almost half of the retail vegetable salad and sprout samples tested were positive for *B. cereus* that contained at least one enterotoxin gene. Additionally, 5.7% of the *B. cereus* isolates were psychrotrophic and could grow at refrigeration temperature of 7°C (Chon et al., 2015).

Yu et al (2019) isolated and analyzed 294 *B. cereus* strains from vegetables in different Chinese towns. 50% of vegetable samples had *B. cereus* with contamination of more than 1,100 MPN per gram, along with more than 95.6 % of the isolated having multi-drug resistance. All of the studies shed light on *B. cereus* contamination in vegetables and emphasize the importance of vegetable handling for food safety (Yu et al., 2019).

Cereal and meat products including cooked rice, pasta have been associated with several *B. cereus* food positioning as listed in Table 1. As a particular concern for contamination by *B. cereus*, and the bacterium is present in ready-to-eat foods with cereal products and has an estimated prevalence of 41.48% and meat products of 11.87% (Rahnama et al., 2023, Lesley et al., 2013). While rice products are the main cause of *B. cereus* poisoning, rice is also one of the most widely consumed cereals worldwide, especially in developing countries. Improper storage of cooked rice and meat in hot weather conditions can significantly increase the risk of food poisoning caused *B. cereus* as the regular thermal treatment is not sufficient to inactivate the spores (Albaridi, 2022). In general, the prevalence of *B. cereus* spores in cooked rice and meat is typically attributed to

slow cooling periods and extended storage at room temperature. These conditions facilitate the germination and proliferation of the spores (Soni et al., 2016).

Importantly, *B. cereus* contamination of meat has been reported not only during slaughter and with raw meat, but also with processed meat. This is due to the prevalence of the bacteria in food production and processing environments (Soleimani et al., 2017). A recent study concluded that poor-quality raw materials were mostly responsible for the extremely high incidence of *B. cereus* in sausages (70% of samples). Because of this, contaminated raw meat is a major contributor to the widespread presence of *B. cereus* in processed meats. Meat, on the other hand, was found to have lower levels of contamination than grains and dairy products, likely because meat is not grown on soil. Maintaining clean slaughtering, transporting, packaging, and storing practices helps keep meat and protein products free of contamination (Oluwafemi et al., 2013).

A selective medium PEMBA was used to examine 102 samples of raw and cooked poultry meat products for the presence of *Bacillus cereus*. With values ranging from 1.4 to 3.5 log CFU/g, *B. cereus* was only detectable in 6.9% of the samples. However, there were occasionallynumerous additional microorganisms present (up to 10^7 cells/g), which may have obscured the presence of *B. cereus*. The presence of other organisms can negatively impact the recovery of *B. cereus*, according to an experiment using three selective media and three standard strains of the bacteria (Sooltan et al., 1987).

Bacillus cereus is presented in many spices and seasonings as well. As meat goes through further processing, the risk of contamination by *B. cereus* rises. The increased *B. cereus* load in the finished products can be traced back in large part to the meat additives that are used in the processing. *B. cereus* levels of up to 500 cells/g were found in processed meats including hamburgers and ground beef (Tewari & Abdullah, 2015; Volkova, 1971).

Moreover, *B. cereus* can be found in frozen foods and other food products. In a study that was carried out by Mira and Abuzied, five different kinds of ready-to-eat chicken products and frozen partially cooked chicken products were obtained from fast food shops and supermarkets, and *B. cereus* was found in all samples. The ready-to-eat chicken products had the highest incidence of *B. cereus*, followed by the frozen half-cooked chicken products, which had the lowest incidence (Ki Mira & Ma Abuzied, 2006).

Biofilms formed by *B. cereus* are also a contamination source of *Bacillus* spores during food processing, leading to spoilage, food poisoning, and compromised food quality. *Bacillus* cells and spores possess the ability to adhere to stainless steel surfaces where they multiply, forming small clusters of cells that secrete extracellular polymeric substances (including the earlier mentioned BclA). Over time, these microcolonies aggregate, developing into hard-to-clean biofilms in areas like storage tanks and processing lines within processing plants, especially in inaccessible and hard-to-clean spots (Shi and Zhu, 2009).

Interestingly, *B. cereus* biofilms can harbor not only spores but also vegetative cells containing spores. These spores within biofilms have been reported to exhibit enhanced resistance to antimicrobials, cleaning procedures, and disinfectants, compared to spores grown in liquid culture (Wirtanen et al., 1996). The detachment and subsequent release of these spores from biofilms could lead to the contamination of other surfaces or food being processed (Faille et al., 2014).

Egg products are reported with the presence of *B. cereus* due to the inclusion of cracked and contaminated eggs, coupled with reduced cleaning and disinfection (Wood and Waites 1988). Additionally, Techer et al., 2014 reported that psychrotrophic *B. cereus* group bacteria mainly present a spoilage risk in the egg product industry by their ability to grow and to induce egg product spoilage at low temperatures. Moreover, in the same risk assessment study, the author points out

that the food safety and food spoilage risks are negatively correlated at the individual level from a principal component analysis. The *B. cereus* strains that display the highest pathogenicity at moderate temperatures tend to cause the least spoilage when stored at refrigerated temperatures (Lesley et al., 2013).

Milk and dairy products:

Contamination of *B. cereus* in milk and dairy products is reported from the source of raw milk and subsequent temperature abuse during storage and transportation (Burgess et al., 2010). *Bacillus cereus* spores can live in pasteurized milk and have the potential to contaminate the milk if the heat used in the pasteurization process is insufficient or low, or if there is contamination after the milk has been pasteurized (Doyle et al., 2020).

Even though milk and dairy products rarely cause *B. cereus* outbreaks, protecting infants who rely on powdered milk-based products from contamination is essential. To reduce the risk of *B. cereus* contamination in milk and dairy products, it is important to properly clean cow udders before to milking and to utilize appropriate procedures to sanitize storage tanks, transport tubes, and processing facilities (Ibrahim et al., 2022). Over the course of five months, researchers in Madison, Wisconsin analyzed 400 samples of milk and milk products from different retail outlets to determine the prevalence and concentration of *Bacillus cereus*. The results showed that *B. cereus* was present in 14% of the Cheddar cheese samples, 9% of the raw milk samples, 35% of the pasteurized milk samples, and 48% of the ice cream samples. Raw milk tested at 100 CFU/ml, pasteurized milk at 1,000 CFU/ml, Cheddar cheese at 200 CFU/g, and ice cream at 3,800 CFU/g (Ahmed et al., 1983). *Bacillus cereus* prevalence in Chilean schools was investigated by testing 381 samples of dried milk products including milk powder and pudding milk. Whole rice, extruded cereals and pulses, and food additives were found to have the highest amounts of

contamination. In addition, psychrotrophic temperature growth and the ability to produce diarrheal enterotoxin were assessed in 94 *B. cereus* isolates. Only 28 (30.8%) of the 94 examined isolates were able to grow at 7 °C or lower, but all of them were positive for the generation of diarrheal enterotoxin. The study indicated that there was a significant incidence of *B. cereus* in dried milk products, with most strains being enterotoxigenic mesophilic strains, which could pose a threat to the safety of reconstituted goods kept at unsuitable temperatures (Ahmed et al., 1983).

PREDICTIVE MICROBIOLOGY AND ITS APPLICATION IN FOOD QUALITY

The microbiological safety of foods is of particular significance. Food safety and quality is a significant concern for public health. Challenge tests, which imitate the impact of external conditions on food by monitoring the growth and multiplication of spoilage and harmful microbes, have traditionally been used to prove the microbiological safety of foods. Challenge tests are useful research tools for establishing the safety and shelf life of food under controlled conditions, but they also have drawbacks, such as being costly, labor-intensive, time-consuming, and non-cumulative (Notermans et al., 1994).

After an extended period of development, predictive microbiology (the quantitative study of microbial ecology in foods) has robustly established itself as a vital component of contemporary food microbiology. In comparison to challenging tests, which are limited by their time-consuming and non-cumulative nature, it provides a more time-efficient, cost-effective, and reliable alternative. For more effective management of food safety and quality, predictive microbiology can provide valuable information on the development and contamination of microorganisms in a variety of situations.

Comparatively, predictive microbiology is a more feasible and economical method for analyzing

how microorganisms behave in diverse environmental conditions (Ross & Dalgaard, 2004). In this method, the proliferation of bacteria in controlled settings, such as temperature, pH, water activity, sodium chloride concentration, and antimicrobials, is measured. The data is thereafter used for predicting the microorganism's proliferation using mathematical, statistical, and microbiological concepts. The behavior of microorganisms under hypothetical settings can then be predicted using the condensed results of these calculations (Baranyi et al., 1996).

A reliable approach for predicting bacterial growth and contamination in various conditions, such as during emergencies or when keeping the cold chain, is predictive microbiology. It can assist in lowering the risk of foodborne illness and spoilage by properly forecasting the bacteria proliferation under certain circumstances. This is particularly crucial during abusive temperature conditions including transporting perishable items or during electrical power outages. Additionally, this method's quantitative and predictive characteristics enable cumulative study, where new data may be added to existing databases to enhance the precision and dependability of forecasts over time (McMeekin et al., 2008).

Two key modeling approaches—kinetic modeling and probability modeling - can be used to examine various microbial processes in the field of predictive microbiology. Kinetic modeling, which encompasses growth models, survival models, and inactivation models, is concerned with estimating the rate and degree of microbial growth or decline. On the other hand, probability modeling calculates the probability that a microbiological event, like toxin formation or viral transmission, will take place. Due to its adaptability and versatility, kinetic modeling is the method that is utilized in the food sector the most frequently. There are three categories of models in kinetic modeling: primary, secondary, and tertiary models (Pérez-Rodríguez et al., 2008). Although there are other models like the Pathogen Modeling Program (PMP) offers a user-friendly interface and

a comprehensive database for modeling pathogen growth in various food products. It allows for the validation and customization of models but may have limited coverage of less common or emerging pathogens. On the other hand, ComBase Predictor provides a large and continuously expanding database of microbial growth data, offering diverse models and integration with the ComBase database. These PMP and ComBase have data base which was developed by conducting experiments in microbiological broth medium, but not validated in food matrices (Juneja et al., 2019). However, it may be more complex for inexperienced users and requires data interpretation. Overall, both programs offer advantages such as access to extensive data and the ability to customize models, but they also have limitations including limited species coverage and potential complexity for certain users.

Primary Modeling

In predictive microbiology, primary modeling is the ground base for estimating kinetic parameters such as maximal growth rate, lag phase, and inactivation rate as a function of treatment time under certain conditions (pH, water activity, temperature, sodium chloride concentration, etc). These estimates are useful for simulating storage phases, processing, or heat treatment in model applications. To estimate values for model parameters like the length of the lag phase, maximum population, or the growth rate for growth models, the observed microbial growth data were fitted to a series of mathematical equations such as Baranyi, Huang, logistic, or modified Gompertz models. Only the intrinsic and extrinsic factors from which the observed dataset was obtained are applicable to the resulting predictive models. Lag, exponential, stationary, and death phases are the four main phases of microbial growth. Sigmoidal functions, including the modified logistic and modified Gompertz models, are commonly used to fit microbial growth data. Additionally, the MATLAB 7.0 curve-fitting tool with 95% confidence limits is often employed for parameter

estimation. However, variations can occur when different methodologies are used to measure growth, leading to differences in fitted parameters. New techniques involving microscopic image analysis have been developed to gain insights into bacterial colony growth dynamics. Moreover, by applying these mathematical models like the Gompertz, Baranyi, and logistic models, which help construct sigmoidal growth curves that illustrate the growth phases of microorganisms, primary models play a crucial part in comprehending these growth phases (Buchanan & Klawitter, 1991).

Secondary Modelling

The estimates from primary model parameters (maximal growth rate, lag phase, and inactivation/growth rate) at numerous environmental elements like temperature, pH, and water activity could be integrated into the secondary model. Therefore, the secondary model can be utilized for predicting the growth/inactivation of microorganisms in various situations because they integrate the data from primary models (Longhi et al., 2013).

The Ratkowsky square root model, which depicts a linear relationship between growth rates and temperature, is one prominent secondary model (Juneja et al., 2008). Other popular secondary models include the Arrhenius and response surface (polynomial) models. The Ratkowsky model provides a biologically relevant explanation for the parameters because it is a mechanistic model. This makes it simpler to comprehend how external factors affect bacterial development and its underlying mechanisms. Polynomial models, on the other hand, are empirical and created based on the statistical analysis of the data (Ratkowsky et al., 1982). Depending on the quantity of environmental parameters, secondary modeling can be approached in one of two ways. In the first method, each environmental factor's impact is modelled separately before a general model is created to describe how they all together affect bacterial behavior. This method is used in

common secondary models like the square root model and cardinal parameter models. The second method uses a polynomial function to simultaneously model the impact of several environmental elements. This method enables the modeling of intricate interactions between numerous components, although the resulting model could be more challenging to understand (Gibson et al., 1987).

Tertiary Modeling

By integrating primary and secondary models, tertiary models are ordinary differential equations (ODE) that are used to forecast microorganism growth under environmental circumstances (usually under temperature abuse). The predicted microbial growth can be calculated from solving the ode from primary and secondary models in software such as MATLAB or R program. However, this process required programming and mathematical skills that are not user friendly for food processors. In comparison to manual calculation in MATLAB or R program., some software is introduced that is more accessible and user friendly. Such software includes the Pathogen Modeling Program (PMP) by the USDA Food Safety Research Unit, which builds multivariant models based on the Gompertz function and response surface analysis using experimental data. However, the customization of PMP is constrained by the restricted access to raw data. The software program ComBase, on the other hand, offers a library of more than 50,000 records of bacterial growth and inactivation in food and culture media. Users can browse, create new modeling strategies, and maintain permanent recordings of experimental data using ComBase, which increases the effectiveness of data analysis. In order to ensure food safety and prevent foodborne illnesses, tertiary modeling is essential since it offers more precise and adaptable forecasts of microbial growth (Baranyi & Tamplin, 2004).

Validation Methods

Due to the data used for predictive modeling is being derived under lab conditions, microbial growth is reported to be overestimated and creating bias in the models (Baranyi et al., 1999). The growth history of the inoculum, naturally occurring food microflora, and internal and extrinsic factors that may affect microbial activity are a few examples of the factors that may cause differences between the predicted and observed growth of microorganisms. Experimental data can be utilized for validation to make sure that predictive models are accurate. The acceptable prediction zone (APZ) method, the bias factor, and the accuracy factor are just a few of the analysis techniques that can be used. The accuracy and bias factors work in combination to provide straightforward indicators of the model's success. These indices determine any bias that can result in inaccurate predictions and evaluate the degree of confidence

in the model's predictions. In other words, they assist in identifying the model's dependability and potential for making inaccurate predictions. Researchers may find and fix any model problems using these metrics, which will increase the model's accuracy and dependability, preventing and avoiding foodborne diseases (Ross, 1996).

Advantages and Disadvantages of Predictive Modeling

The three-phase linear model, which is a straightforward and reliable primary model, performed exceptionally well when compared to other models. It yielded growth kinetics values that were similar to those obtained using the Gompertz and Baranyi models, and all three models had comparable "quality of fits." When the model is applicable to the growth scenario being evaluated (e.g., when there are modest variations in microbial population), it offers several advantages due to its simplicity and flexibility.

The model introduces two elements that help balance the behavior of bacteria as populations and individual cells. These elements are: 1) the consideration of biological variation and 2) the division

of the lag time into two periods. By directly observing individual cells, it becomes possible to calculate the variance of the lag time (tLAG) and the growth rate (μ) in the case of biological variation.

Unlike the Gompertz and Baranyi models, but similar to the reparameterization of the Gompertz model by Zwietering et al. (1990), the three-phase linear model has a distinct term for tLAG. This feature offers clear advantages in terms of accounting for the impact of cultural history on the adaptation time.

For estimating bacterial growth kinetics, the three-phase linear model proves a straightforward and efficient primary model that can be easily applied using curve fitting software. However, it is important to accurately describe the difference between the lag and exponential phases (Breidt et al., 2013).

In the context of food shelf-life prediction and quantitative risk analysis of food production cycles, the Baranyi model stands out as a truly dynamic model due to its ability to handle time-varying environmental conditions (Yılmaz & Cikrikcioglu, 2011). However, a critical assumption of this model is the constant correlation between "lag time" and "maximum specific growth rate." Furthermore, the shape of the growth curve does not provide sufficient details to accurately quantify the physiological effects of alterations (Peleg, 2006). The Baranyi model is also inadequate for development patterns with lengthy "logarithmic lag times," during which the growth rate effectively becomes zero for a significant period (Peleg & Corradini, 2011).

The Gompertz model has always been purely phenomenological in its description. It can represent growth curves with various lag times, whether short, long, or nonexistent, by adjusting its parameters. It has primarily been used in food microbiology to fit isothermal growth curves or to analyze data under constant environmental conditions. Once the coefficients' dependence on

temperature or other parameters is empirically determined and algebraically represented, the Gompertz model can be transformed into a rate model. However, there is a limitation if the model is converted into a rate equation and utilized for dynamic growth forecasting or establishing a boundary condition (Peleg & Corradini, 2011).

Conclusion

B. cereus has been associated with human infections which are rare in occurrence, but this is a ubiquitous and coexists with the usual flora in humans. This has evolved a plethora of virulent characteristics which enables the pathogen's ability to breach the anatomical barriers leading to the disease. The presence of this bacterium is inevitable in the presence of storage or other abuses which trigger their growth. This should be researched to allow the food industry to set the standards for abating the food safety concerns from *B. cereus* in various conditions in different types of foods. This can be achieved by predictive modeling which protects the food safety standards from unforeseen temperature deviations. Finally, *B. cereus* being a resilient bacteria should be considered with great importance and further outbreaks can be ameliorated by setting up the standards for a variety of foods that might be potentially involved using reliable models.

Table 2.1: *B. cereus* outbreaks and the foods associated with the food poisoning from *B. cereus* contamination.

Type of food	Epidemiology	Reference
Fresh vegetables	With a prevalence of 36.94 % due to contact with the soil. Raw vegetables found with 47 % contamination (102 samples). 50 % of vegetable samples found with contamination with more than 95.6 % multi drug resistance.	Rahnama et al., 2023 Park et al., 2018 Yu et al., 2019
Ready-to-eat Cereal products	Prevalence of 41.48%.	Rahnama et al., 2023
Cooked rice	Prevalence of more than 98% (70 samples) 10^4 CFU/g in 1 out of 70 samples.	Dong, 2013
Pasta and pasta salad	~10^9 and 10^8 detected respectively which were consumed after 3 and 5 days after exposure to intermittent temperature abuse.	Derick et al., 2005 Naranjo et al., 2011
Lentil soup	Detected ~10^4 CFU/g in stool samples of affected people. This was suspected to be contaminated from turmeric powder.	NZPHSR, 2007
Meat products		
Cooked poultry products	6.9 % of the samples ranging from 1.4 to 3.5 log CFU/g.	Lesley et al., 2013
Hamburgers and ground beef	Detected about 500 CFU/g.	Tewari & Abdullah, 2015
Pork	Detected ~ 10^5 CFU/g due to improper refrigeration.	Luby et al., 1993
Milk products		
Cheddar cheese	Prevalence of about 14%, tested at 200 CFU/g.	Ibrahim et al., 2022 Ahmed et al., 1983
Raw milk	Prevalence of about 9%, tested at 100 CFU/ml.	Ibrahim et al., 2022 Ahmed et al., 1983
Pasteurized milk	Prevalence of about 35%, tested at 1000 CFU/ml.	Ibrahim et al., 2022 Ahmed et al., 1983
Ice cream	Prevalence of about 48%, tested at 3800 CFU/g.	Ibrahim et al., 2022 Ahmed et al., 1983

Dried milk products	Prevalence of about 44.9%, tested at 3800 CFU/g.	Ahmed et al., 1983

References

Agata, N., Ohta, M., & Mori, M. (1996). Production of an emetic toxin, cereulide, is associated with a specific class of *Bacillus cereus*. *Current Microbiology*, *33*, 67–69.

Ahmed, A. A. H., Moustafa, M. K., & Marth, E. H. (1983). Incidence of *Bacillus cereus* in milk and some milk products. *Journal of Food Protection*, *46*(2), 126–128.

Albaridi, N. (2022). Risk of *Bacillus cereus* contamination in cooked rice. *Food Science and Technology*, *42*.

Andersson, A., Granum, P. E., & Roenner, U. (1996). The adhesion of *Bacillus cereus* spores to epithelial cells, at possible virulence mechanism. *Food Associated Pathogens, Uppsala (Sweden), 6-8 May 1996*.

Augustin, J.-C. (2011). Challenges in risk assessment and predictive microbiology of foodborne spore- forming bacteria. *Food Microbiology*, *28*(2), 209–213.

Baranyi, J., Ross, T., McMeekin, T. A., & Roberts, T. A. (1996). Effects of parameterization on the performance of empirical models used inpredictive microbiology'. *Food Microbiology*, *13*(1), 83– 91.

Baranyi, J., & Tamplin, M. L. (2004). ComBase: a common database on microbial responses to food environments. *Journal of Food Protection*, *67*(9), 1967–1971.

Beecher, D. J., Schoeni, J. L., & Wong, A. C. (1995). Enterotoxic activity of hemolysin BL from

Bacillus cereus. *Infection and Immunity*, *63*(11), 4423–4428.

Buchanan, R. L., & Klawitter, L. A. (1991). Effect of temperature history on the growth of *Listeria monocytogenes* Scott A at refrigeration temperatures. *International Journal of Food*

Microbiology, *12*(2–3), 235–245.

Carlin, F., Fricker, M., Pielaat, A., Heisterkamp, S., Shaheen, R., Salonen, M. S., Svensson, B., Nguyen- the, C., & Ehling-Schulz, M. (2006). Emetic toxin-producing strains of *Bacillus cereus* show distinct characteristics within the *Bacillus cereus* group. *International Journal of Food Microbiology*, *109*(1–2), 132–138.

Cho, W.-I., & Chung, M.-S. (2020). *Bacillus* spores: A review of their properties and inactivation processing technologies. *Food Science and Biotechnology*, *29*, 1447–1461.

Chon, J.-W., Yim, J.-H., Kim, H.-S., Kim, D.-H., Kim, H., Oh, D.-H., Kim, S.-K., & Seo, K.-H. (2015).

Quantitative prevalence and toxin gene profile of *Bacillus cereus* from ready-to-eat vegetables in South Korea. *Foodborne Pathogens and Disease, 12*(9), 795–799.

Cliver, D. O., & Cochrane, B. A. (1986). *Progress in Food Safety: Symposium Entitled" Progress in Our Knowledge of Foodborne Disease During the Life of the Food Research Institute"*. Food Research Institute, Wisconsin University.

DeBuono, B. A., Brondum, J., Kramer, J. M., Gilbert, R. J., & Opal, S. M. (1988). Plasmid, serotypic, and enterotoxin analysis of *Bacillus cereus* in an outbreak setting. *Journal of Clinical Microbiology*, *26*(8), 1571–

1574.

Diplock, K. (2023). *Foodborne illness outbreaks: Roles and responsibilities*.

Doyle, M. P., Diez-Gonzalez, F., & Hill, C. (2020). *Food microbiology: fundamentals and frontiers*.

John Wiley & Sons.

Drobniewski, F. A. (1993). *Bacillus cereus* and related species. *Clinical Microbiology Reviews*, *6*(4), 324–338.

Ehling-Schulz, M., Guinebretiere, M.-H., Monthán, A., Berge, O., Fricker, M., & Svensson, B. (2006). Toxin gene profiling of enterotoxic and emetic *Bacillus cereus*. *FEMS Microbiology Letters*, *260*(2), 232–240.

Ehling-Schulz, M., Knutsson, R., & Scherer, S. (2010). *Bacillus cereus*. *Genomes of Foodborne and Waterborne Pathogens*, 147–164.

Ehling-Schulz, M., & Messelhäusser, U. (2012). One pathogen but two different types of foodborne outbreak: *Bacillus cereus* in catering facilities in Germany. In *Case Studies in Food Safety and Authenticity* (pp. 63–70). Elsevier.

Fimlaid, K. A., & Shen, A. (2015). Diverse mechanisms regulate sporulation sigma factor activity in the Firmicutes. *Current Opinion in Microbiology*, *24*, 88–95.

Foods, I. C. on M. S. for. (1996). *Microorganisms in foods 5: Characteristics of microbial pathogens*

(Vol. 5). Springer Science & Business Media.

Gibson, A. M., Bratchell, N., & Roberts, T. A. (1987). The effect of sodium chloride and temperature on the rate and extent of growth of *Clostridium botulinum* type A in pasteurized pork slurry. *Journal of Applied Microbiology*, *62*(6), 479–490.

Ibrahim, A. S., Hafiz, N. M., & Saad, M. F. (2022). Prevalence of *Bacillus cereus* in dairy powders focusing on its toxigenic genes and antimicrobial resistance. *Archives of Microbiology*, *204*(6), 339.

Jenson, I., & Moir, C. J. (2003). *Bacillus cereus* and other *Bacillus* species. *Foodborne Microorganisms of Public Health Significance, Ed. 6*, 445–478.

Juneja, V. K., Marks, H., & Thippareddi, H. (2008). Predictive model for growth of *Clostridium perfringens* during cooling of cooked uncured beef. *Food Microbiology*, *25*(1), 42–55.

Juneja, V. K., Mishra, A., & Pradhan, A. K. (2017). Dynamic predictive model for growth of

Bacillus cereus from spores in cooked beans. *Journal of Food Protection*, *81*(2), 308–315.

Ki Mira, E., & Ma Abuzied, S. (2006). Prevalence of *Bacillus cereus* and its enterotoxin in some cooked and half cooked chicken products. *Assiut Veterinary Medical Journal*, *52*(109), 70–78.

Kotiranta, A., Lounatmaa, K., & Haapasalo, M. (2000). Epidemiology and pathogenesis of *Bacillus cereus* infections. *Microbes and Infection*, *2*(2), 189–198.

Kramer, J. M., & Gilbert, R. J. (1989). *Bacillus cereus* and other Bacillus species. *Foodborne Bacterial Pathogens*, *19*, 21–70.

Lesley, M. B., Velnetti, L., Yousr, A. N., Kasing, A., & Samuel, L. (2013). The presence of *Bacillus cereus* sl from ready-to-eat cereals (RTE) products in Sarawak. *International Food Research Journal*, *20*(2).

Longhi, D. A., Dalcanton, F., de Aragão, G. M. F., Carciofi, B. A. M., & Laurindo, J. B. (2013). Assessing the prediction ability of different mathematical models for the growth of *Lactobacillus plantarum* under non-isothermal conditions. *Journal of Theoretical Biology*, *335*, 88–96.

Luby, S., Jones, J., Dowda, H., Kramer, J., & Horan, J. (1993). A large outbreak of gastroenteritis caused by diarrheal toxin-producing *Bacillus cereus*. *Journal of Infectious Diseases*, *167*(6), 1452– 1455.

Lund, T., & Granum, P. E. (1996). Characterisation of a non-haemolytic enterotoxin complex from

Bacillus cereus isolated after a foodborne outbreak. *FEMS Microbiology Letters*, *141*(2–3), 151–156. https://doi.org/10.1111/j.1574-6968.1996.tb08377.x

McMeekin, T., Bowman, J., McQuestin, O., Mellefont, L., Ross, T., & Tamplin, M. (2008). The future of predictive microbiology: Strategic research, innovative applications and great expectations. *International Journal of Food Microbiology*, *128*(1), 2–9. https://doi.org/https://doi.org/10.1016/j.ijfoodmicro.2008.06.026

Mogren, L., Windstam, S., Boqvist, S., Vågsholm, I., Söderqvist, K.,

Rosberg, A.-K., Darlison, J., Mulaosmanovic, E., Karlsson, M., Uhlig, E., Håkansson, Å., & Alsanius, B. (2018). The Hurdle Approach–A Holistic Concept for Controlling Food Safety Risks Associated with Pathogenic Bacterial Contamination of Leafy Green Vegetables. A Review. *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.01965.

Mols, M., & Abee, T. (2011). Primary and secondary oxidative stress in *Bacillus*. *Environmental Microbiology*, *13*(6), 1387–1394.

Notermans, S., & in't Veld, P. (1994). Microbiological challenge testing for ensuring safety of food products. *International Journal of Food Microbiology*, *24*(1), 33–39. https://doi.org/https://doi.org/10.1016/0168-1605(94)90104-X

Oluwafemi, R. A., Edugbo, O. M., Solanke, E. O., & Akinyeye, A. J. (2013). Meat quality, nutrition security and public health: a review of beef processing practices in Nigeria. *African Journal of Food Science and Technology*, *4*(5), 96–99.

Park, K. M., Jeong, M., Park, K. J., & Koo, M. (2018). Prevalence, Enterotoxin Genes, and Antibiotic Resistance of *Bacillus cereus* Isolated from Raw Vegetables in Korea. *Journal of Food Protection*, *81*(10), 1590–1597. https://doi.org/https://doi.org/10.4315/0362-028X.JFP-18-205

Pérez-Rodríguez, F., Valero, A., Carrasco, E., García, R. M., & Zurera, G. (2008). Understanding and modelling bacterial transfer to foods: a review. *Trends in Food Science & Technology*, *19*(3), 131–

144. https://doi.org/https://doi.org/10.1016/j.tifs.2007.08.003

Pielaat, A., Fricker, M., Nauta, M. J., & van Leusden, F. M. (2005). Biodiversity in *Bacillus cereus* RIVM report 250912004/2005. *National Institute for Public Health and the Environment*, *3720*, 1–84.

Centers for Disease Control and Prevention. (2016). Burden of foodborne illness: findings. Centers for Disease Control and Prevention. Available online: https://www. cdc. gov/foodborneburden/2011- foodborne-estimates.

Rajkowski, K. T., & Bennett, R. W. (2003). *Bacillus cereus*. In *International handbook of foodborne pathogens* (pp. 47–60). CRC Press.

Ratkowsky, D. A., Olley, J., McMeekin, T. A., & Ball, A. (1982). Relationship between temperature and growth rate of bacterial cultures. *Journal of Bacteriology*, *149*(1), 1–5.

Ross, T. (1996). Indices for performance evaluation of predictive models in food microbiology. *Journal of Applied Bacteriology*, *81*(5), 501–508. https://doi.org/10.1111/j.1365-2672.1996.tb03539.x

Ross, T., & Dalgaard, P. (2004). Secondary models. *Modeling Microbial Responses in Food*, *17*, 360. Satapute, P. P., Olekar, H. S., Shetti, A. A., Kulkarni, A. G., Hiremath, G. B., Patagundi, B. I., Shivsharan, C. T., & Kaliwal, B. B. (2012). Isolation and characterization of nitrogen fixing *Bacillus subtilis* strain as-4 from agricultural soil. *International Journal of Recent Scientific Research*, *3*(9), 762–765.

Shinagawa, K., Konuma, H., Sekita, H., & Sugii, S. (1995). Emesis of rhesus monkeys induced by intragastric administration with the HEp-2 vacuolation factor (cereulide) produced by *Bacillus cereus*. *FEMS Microbiology Letters*, *130*(1), 87–90.

Soleimani, M., Hosseini, H., Neyestani, Z., Siadati, S., & Pilevar, Z. (2017). Occurrence of *Bacillus cereus* in beef burger marketed in Tehran, capital of Iran. *Journal of Food Quality and Hazards Control*, *4*(3), 70–73.

Sooltan, J. R. A., Mead, G. C., & Norris, A. P. (1987). Incidence and growth potential of *Bacillus cereus* in poultrymeat products. *Food Microbiology*, *4*(4), 347–351.

Stenfors Arnesen, L. P., Fagerlund, A., & Granum, P. E. (2008). From soil to gut: *Bacillus cereus* and its food poisoning toxins. *FEMS Microbiology Reviews*, *32*(4), 579–606.

Tewari, A., & Abdullah, S. (2015). *Bacillus cereus* food poisoning: international and Indian perspective. *Journal of Food Science and Technology*, *52*, 2500–2511.

Turnbull, P. C. B., & Kramer, J. M. (1985). Intestinal carriage of *Bacillus cereus*: faecal isolation studies in three population groups. *Epidemiology & Infection*, *95*(3), 629–638.

Vilain, S., Luo, Y., Hildreth, M. B., & Brözel, V. S. (2006). Analysis of the life cycle of the soil saprophyte *Bacillus cereus* in liquid soil extract and in soil. *Applied and Environmental Microbiology*, *72*(7), 4970–4977.

Volkova, R. S. (1971). *Bacillus cereus* contamination of foods and environment at institutional feeding points. *Gigienai-Sanitariya*, *36*(2), 108–109.

Wijnands, L. M. (2008). *Bacillus cereus associated food borne disease quantitative aspects of exposure assessment and hazard characterization*. Wageningen University and Research.

Yu, P., Yu, S., Wang, J., Guo, H., Zhang, Y., Liao, X., Zhang, J., Wu, S., Gu, Q., & Xue, L. (2019).

Bacillus cereus isolated from vegetables in China: incidence, genetic diversity, virulence genes, and antimicrobial resistance. *Frontiers in Microbiology*, *10*, 948.

CHAPTER 3

DEVELOPMENT AND VALIDATION OF DYNAMIC PREDICTIVE MODEL FOR THE GROWTH FOR BACILLUS CEREUS IN READY-TO-EAT (RTE) TURKEY BREAST[1]

[1]Sujitha Bhumanapalli *et al.* To be submitted to *Journal of Food Protection*

Abstract

Bacillus cereus is a ubiquitous spore-producing foodborne pathogen often isolated from raw food ingredients and occasionally in processed foods and can cause diarrheal and/or emetic illnesses in humans. A dynamic model for predicting *B. cereus* growth in RTE turkey breast was developed and validated using varying dynamic (sinusoidal temperature) conditions. Turkey breast was ground and mixed with a marinade containing water, salt, and phosphate (10.0, 1.0 and 0.3% of the finished product, respectively). Formulated turkey (5 g) was portioned into vacuum bags and inoculated with a four-strain cocktail of *B. cereus* spores to obtain ca. 2.5 log CFU/g. *B. cereus* growth data at isothermal temperatures (10, 15, 20, 25, 30, 35, 37, 39, 41, 43, 45, and 55ºC) were collected. The Baranyi model was used as a primary model to fit growth data; then the growth rate was fitted using the Ratkowsky model (secondary model) as a function of temperature. The mean RMSE and R^2 values for the primary model were 0.2966 and 0.9918, and for the secondary model were 0.0260 and 0.9805, respectively. A tertiary dynamic model was developed using the 4^{th}-order Runge-Kutta method. The dynamic model was validated using two sinusoidal temperature profiles, 10-40°C for 35 h and 5-15°C for 27 days. The accuracy and bias factors were 1.14 and 1.13 for 10-40°C; and 1.07 and 0.94 for 5-15°C, respectively, which were within the acceptable range (0.75 – 1.25). The APZ analysis for validation indicated that >98% of the predicted errors were within the acceptable prediction zone (-1.00 to 0.50 log CFU/g). The developed model can be used to evaluate the growth of *B. cereus* in turkey RTE breast during processing (stabilization), or extended storage and distribution.

Introduction

Bacillus cereus is a gram-positive, facultative anaerobic, motile (flagellated), rod-shaped and spore-forming bacterium (Kotiranta et al., 2000). The microorganism is widely distributed in the

environment and frequently isolated from meat and meat products, processed foods including rice, milk, dairy products, raw and ready-to-eat vegetables, cereals, spices, legumes, and pasta, among others (Eglezos et al., 2010; Ellouze et al., 2021; Esteban-Cuesta et al., 2018; Juneja et al., 2017; Juneja, Golden, Mishra, Harrison, Mohr, et al., 2019; Wijnands et al., 2006). *B. cereus* causes two distinct types of food poisoning: emetic (vomiting) and diarrheal (Granum, 1994; Messelhäußer and Ehling-Schulz, 2018). Symptoms for the emetic type typically manifest within 1-5 h subsequent to consumption of food contaminated with the pre-formed toxin. While for the diarrheal type, symptoms are elicited 8-16 h following the consumption of contaminated food due to ingestion of a large number of vegetative cells. The United States Centers for Disease Control and Prevention (CDC) estimated that 63,400 cases of foodborne illness and 20 hospitalizations occur annually in the United States due to *B. cereus* (Scallan et al., 2011).

Meat and poultry products are generally implicated in most of the outbreaks of *B. cereus* foodborne illness (Bennett et al., 2013). In 1977, a diarrheal outbreak associated with *B. cereus* in turkey loaf led to the hospitalization of 28 symptomatic patients (Giannella and Brasile, 1979). *B. cereus* is a common contaminant of spices used in the production of cooked RTE turkey products (Cufaoglu and Ayaz, 2022). However, most of the *B. cereus* foodborne illness cases are not reported as the symptoms are mild. Emetic illness outbreaks are primarily linked with the consumption of starchy foods, while diarrheal food poisoning is usually associated with the consumption of foods containing meat and vegetables (Griffiths and Schraft, 2017).

The U.S. Department of Agriculture, Food Safety, and Inspection Service (USDA FSIS) requires that turkey products be cooked to a safe minimum internal temperature of 165°F (73.89°C) to ensure the destruction of *Salmonella* and other vegetative foodborne pathogens (USDA, 2010; and Juneja et al., 2020). However, *B. cereus* endospores can survive such heat treatments, are activated

and can out-grow during abusive cooling. The *B. cereus* diarrheal toxin is heat sensitive (56°C for 5 min), but the emetic toxin is heat stable and can withstand high cooking temperatures (126°C for 90 min; Carlin et al., 2006). Meanwhile, the pathogen can grow and produce toxins at temperatures between 5°C and 57°C, considered the danger zone (FDA, 2013). Therefore, food storage at improper temperatures for several hours before serving or inadequate rapid cooling of food after high-temperature cooking can lead to foodborne illness outbreaks from meat products (Adams and Moss, 2000; Mossel et al., 1991).

Predictive growth pathogens under varying time-temperature conditions across the food supply chain. The growth models for *B. cereus* are available in ComBase and Pathogen Modeling Program (PMP; United States Department of Agriculture, Agricultural Research Service). However, predictions from these models can be obtained for the static temperature conditions and were developed using a liquid microbiological medium (Kang et al., 2010). Further, these models have not been validated for foods and do not accurately predict the growth of *B. cereus* on turkey products across the entire biokinetic range. Currently, three dynamic predictive models are available in the literature for the growth of *B. cereus* spores in cooked beans, rice, and pasta (Juneja et al., 2017; Juneja et al., 2019). There is a need for dynamic models for *B. cereus* growth in meat and poultry products. The objective of this research was to develop and validate a dynamic model for estimating the growth of *B. cereus* spores in RTE turkey breast. The developed predictive model can be helpful for regulatory agencies such as USDA-FSIS and meat processing plants to address the public health concern about the production of *B. cereus* emetic and diarrheal toxins in RTE turkey breast under conditions of temperature abuse due to inadequate cooling rate or improper temperature conditions during storage.

Methods and materials

Bacillus cereus strains

Four strains of *Bacillus cereus* were obtained from the Center for Food Safety, UGA. All the four strains are diarrheal toxin producing strains which include *B. cereus* F4810/72, isolated from cooked rice, *B. cereus* F4512A/87 isolated from pasteurized milk, *B. cereus* 038-2 isolated from infant formula, and *B. cereus* F3812/84 isolated from pasteurized milk. The strains were maintained as stock culture in cryobeads (Pro-Lab Diagnostics Microbank™, PL.170C/G, Thermo Fisher Scientific, USA) until use at -80ºC.

B. cereus Spore preparation

B. cereus spores were prepared as described by Juneja et al., (2017) Briefly, a bead of each strain was inoculated into sterile brain heart infusion broth (10 mL, BHI) and incubated overnight at 37°C. Subsequently, 100 µl of each cell suspension was surface plated on thirty NAMS (thirty nutrient agar (Difco, BD, Sparks, MD and Fisher Scientific) containing manganese sulfate (0.05 g/L MnSO4; Waltham, Massachusetts, USA) agar plates. The NAMS agar plates were incubated for 10 d at 37°C to generate spores (Juneja et al., 2018). Sterile distilled water (5 mL) was added to each NAMS agar plate, and the spores were carefully scraped using sterile plastic spreaders. The spore crop from all 30 plates was collected into Falcon tubes (50 mL; Falcon® Centrifuge Tube, Conical Bottom, Corning, USA) and centrifuged at 10,000 x *g* for 15 min at 4°C (Centrifuge 5804, Eppendorf, Hamburg, Germany). The supernatant was discarded, and the pellet was resuspended in sterile distilled water (10 mL) and centrifuged as described. This process was repeated twice. The pellet was finally resuspended in sterile distilled water (10 mL) and stored at -20°C until further use. The concentration of each spore stock was determined by heat treating the spores for 10 min at 80°C, serially diluted using peptone water (0.1% w/v; PW) and plated on tryptic soya agar (TSA; Difco, Detroit, Michigan, USA) with yeast extract agar (6 g/L; BBL,

Farnklin Lakes, New Jersey, USA). The plates were incubated for 24 h at 37°C and colonies were enumerated. A four-strain spore cocktail of *B. cereus* was prepared by mixing equal populations of each strain of the spore crop.

Sample preparation

The vacuum-packaged turkey breast was shipped under refrigeration and sourced from a commercial turkey processor. The turkey breast was diced into ca. 2.5 cm cubes and ground using a 19 mm grinder plate using a meat grinder (PC–98/32, Mainca USA Inc., St. Louis, Missouri, USA). The ground turkey was placed in a meat mixer (RM – 20 INT s/n RM20213517, Mainca USA Inc., St. Louis, Missouri, USA) and mixed for 15 min while adding a marinade consisting of 10% water, 1% salt (Custom blended seasonings, A. C. Legg, Inc., Calera, Alabama, USA) and 0.3% phosphate (Sodium polyphosphate, Brifisol® 512, Ettlinger Corporation, Kansas, USA) by weight of the meat block. The marinated meat was subsequently finely ground using 6.35 mm plate (PC–98/32, Mainca USA Inc., St. Louis, Missouri, USA), vacuum-sealed (Multivac Sepp Haggenmuller GmbH and Co. KG, 87787 Wolferrschwenden, Germany) and stored frozen at -20°C until use.

The prepared ground turkey meat was portioned (5 g) into vacuum bags (Ultravac Chamber Vacuum Packaging Pouches 3 mil, UltraSource LLC, Kansas City, Missouri, USA). Each bag was inoculated with 50 µL of *B. cereus* spore cocktail to achieve approximately 2.5 log CFU/g of spores. The air from the bags was removed by carefully pressing the meat and heat sealed (Impulse heat sealer, AIE, Long Beach, California, USA). The sealed samples were mixed gently for about 2 min to ensure uniform distribution of the spores. Non-inoculated samples were used as negative controls. The bags containing the inoculated meat were heat treated for 10 min at 80°C in a circulating water bath (Model MX20H135-A11B Heated Circulating Water Bath, PolyScience,

Illinois, USA) and transferred to water baths maintained at specific temperatures (Juneja et al., 2018).

B. cereus growth at isothermal and sinusoidal profiles

B. cereus growth data was collected at various isothermal temperatures by submerging the heat-treated meat in circulating water bath (MX20H135-A11B Heated Circulating Water Bath, MX Controller, PolyScience, Illinois, USA) set to temperatures ranging from 10 to 55°C, which included the entire biokinetic growth range of *B. cereus* (Ellouze et al., 2021). A sample was removed at each pre-determined time point, and the *B. cereus* population was enumerated. The sample collection period ranged from 40 d at 10°C to 32 h at 30°C to 15 d at 55°C.

To validate the findings, two non-isothermal dynamic profiles were used, and *B. cereus* growth data was collected. The turkey meat pouches were heat-treated as described and transferred to a programmable refrigerated water bath (AP15R-40-A11B, Polyscience, Illinois, USA). The refrigerated water bath was programmed to follow a sinusoidal temperature profile, ranging from 10 to 40°C (5 h cycle) or 5 to 15°C (96 h cycle) for 36 h and 27 d, respectively (Singh et al., 2011). Samples were removed at predetermined intervals and *B. cereus* population was determined. Three independent replications (~15 samples/replication) were performed at each temperature profile.

Enumeration of _B. cereus_ populations

The meat samples were removed at each sampling point and the meat was aseptically transferred to a filter bag (B01385, Whirl-Pak Filter bag, Nasco, Fort Atkinson, Wisconsin, USA) and PW (20 mL) was added. The meat was stomached (Basic paddle maxicator, Neutec Group Inc., New York, USA) for 2 min, serially diluted in PW and plated on Petrifilm™ APC (3M, St. Paul, MN). Negative controls (non-inoculated samples) confirmed the absence of naturally occurring *B. cereus* (Juneja et al., 2019).

pH and water activity (a_w)

The pH and water activity (a_w) of prepared turkey were determined using a calibrated pH meter (pHTestr® 5F, Oakton, Long Beach, New Jersey, USA) and an a_w meter (Dew Point Water Activity Meter 4TE, AquaLab, Pullman, Washington, USA), respectively following manufacturer's instructions.

PREDICTIVE MODELLING

Primary Model

The Baranyi model is commonly used to describe the growth kinetics of microorganisms under various external conditions such as temperature (Juneja et al., 2018). The Baranyi model was constructed for the growth of *B. cereus* as a function of time for each temperature. The model consisted of two differential equations (Equation 1.1 and 1.2; Baranyi and Roberts, 1994)

$$\frac{dy}{dt} = \frac{1}{1+e^{-Q\mu_{max}}}\left(1 - e^{(y-y_{max})}\right) \quad \text{.....(1.1)}$$

$$\frac{dQ}{dt} = \mu_{max} \quad \text{.....(1.2)}$$

These equations had initial conditions of $y = y_0$ at $t = 0$ and $Q = \log_e(q_0)$, where q represents the initial physiological state of the microbial cell populations.

The Baranyi model with four parameters was used for fitting the bacterial growth under isothermal conditions by solving two equations.

$$y^{(t)} = y_0 + \mu_{max}F(t) - \log_e\left(1 + \frac{e^{\mu_{max}F(t)}-1}{e^{(y_{max}-y_0}}\right) \quad \text{.....(2)}$$

Where $F(t) = t + \frac{1}{v}\log_e\left(e^{-vt}+e^{-h_0}-e^{(-vt-h_0)}\right)$ .....(3)

The variable y(t) represents the natural logarithm of the *B. cereus* concentration in CFU/g at time. The initial and maximum *B. cereus* population are denoted by y_0 and y_{max}, in units of ln

CFU/g. The maximum specific growth rate (μ_{max}; ln CFU/-h) is assumed to be equal to the rate of increase of the limiting, ν.

The parameter $h_0 = (\mu_{max}) * (\lambda)$, where λ is the duration of the lag phase in h (Lag time), and is calculated from the following equation:

$$\lambda = \frac{h_0}{\mu_{max}} \quad \text{.....(4)}$$

The results of the model were analyzed and fitted to growth data using ln CFU/mL units and reported in log CFU/mL units (Singh et al., 2011).

The growth data was plotted against time, and the initial values of the parameters such as h_0, y_0, y_{max} and μ_{max} were estimated at each temperature point. The starting values for y_0 and y_{max} were provided as the lower and upper asymptotes, respectively. The maximum growth rate (μ_{max}) was determined based on the slope of the exponential phase of the bacterial count over time. The starting value (h_0), was determined by multiplying lag time (λ) and maximum growth rate (μ_{max}). Lag time refers to the duration between the initiation of growth and the onset of exponential growth (Singh et al., 2011).

Growth data from each temperature point was fitted into the Curve fitting tool in MATLAB software (Version R2018a, MathWorks, Natick, MA) to depict the sigmoidal growth of *B. cereus* (Thomas et al., 2019).The parameter h_0 should remain constant when the pre-inoculation history of bacterial cells is the same (Baranyi and Roberts, 1994). Therefore, the initial estimation of the Baranyi model involved the determination of four parameters: h_0, y_0, y_{max} and μ_{max}. The mean h_0 value was calculated from the models and set as the constant. The other three parameters (y_0, y_{max} and μ_{max}) were estimated again by re-fitting into Baranyi model (Juneja et al., 2007).

Secondary Model

The relationship between the estimates of μ_{max} obtained from the primary Baranyi model and

corresponding temperatures was determined by fitting these parameters as a function of temperature using secondary model (Thomas et al., 2019). The secondary model describes all the biotic and abiotic variables that can change the kinetics of microbial growth, including temperature, water activity, pH, and other elements (Wijtzes et al., 2001, Baranyi and Roberts, 1994; Koutroumanidis et al., 2006). In this case, only temperature was the variable factor, with the product water activity, pH and other elements remaining the same as the same product was used. The secondary Ratkowsky model (Ratkowsky et al., 1983) was used to fit the maximum specific growth rates μ_{max}, to determine the effect of temperature on μ_{max} (Zwietering et al., 1991).

$$\mu_{max}=a(T- T_{min})^2 \{1-\exp[b(T- T_{max})]\} \quad (5)$$

where T is the temperature (ºC), T_{min} and T_{max} are the theoretical minimum and maximum growth limits (ºC) respectively, a and b are the coefficients of regression. The lag time for each temperature was modeled by fitting each temperature lag time using a hyperbolic function (Zwietering et al., 1991).

$$\lambda = \frac{p}{e^{T-q}} \quad (6)$$

where p is a variable that accounts for the decrease in the lag time with an increase in temperature, q is the temperature (ºC) where the lag phase is infinite, and T is the temperature in (ºC).

MATLAB software (Version R2018a, MathWorks, Natick, MA) was used to construct secondary models by using curve fitting toolbox with trust-region algorithm for non-linear least squares (Juneja, Golden, Mishra, Harrison, Mohr, et al., 2019).

Goodness of fit statistics for primary and secondary models

The performance of the primary and secondary models was evaluated by using goodness of fit

statistics. Accuracy and bias factors (Ross, 1996), coefficient of determination (R^2) and root mean square error (RMSE) (Hodson, 2022) were used to compare both model performances, as well as the developed dynamic model (Juneja, Golden, Mishra, Harrison, Mohr, et al., 2019). R^2 was calculated using the equation below (Di Bucchianico, 2008):

$$R^2 = \frac{SSR}{SSTO} \quad (7)$$

Where $SSR = \sum_{i=1}^{n} \left(\hat{y}_i - \bar{y}\right)^2$ (7.1)

and $SSTO = \sum_{i=1}^{n} \left(y_i - \bar{y}\right)^2$ (7.2)

The terms SSR and SSTO are the sum squared regression and total sum of squares, respectively. The goodness of fit statistics was calculated using MATLAB.

The RMSE value was calculated by the given equation below (Chai and Draxler, 2014):

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n} e_i^2} \quad (8)$$

where n is the sample proportion, $\hat{y}$ is the predicted regression, $\overline{y}$ is the mean response parameter, y is response variable (*B. cereus* population density)

$$\text{Accuracy factor} = 10^{\frac{\sum\left|\log\left(\frac{GT_{predicted}}{GT_{observed}}\right)\right|}{n}} \quad (9)$$

$$\text{Bias factor} = 10^{\frac{\sum \log\left(\frac{GT_{predicted}}{GT_{observed}}\right)}{n}} \quad (10)$$

where $GT_{predicted}$ indicates the predicted generation time (h), $GT_{observed}$ indicates the observed generation time (h) and n indicates the sample size.

Tertiary Model

MATLAB software was used to generate tertiary models by combining the primary and secondary models. (Baranyi and Roberts, 1994) suggested that the two first-order differential equations represent differential growth in the model.

$$\frac{dy}{dt} = \frac{1}{1+e^{-Q(t)}} \mu_{max} [T(t)] \left(1-e^{y(t)-y_{max}}\right) \quad \text{.....(11)}$$

$$\frac{dQ}{dt} = \mu_{max} [T(t)] \quad \text{.....(12)}$$

where $Q(t)=\ln q(t)$

where $y(0)=y_0$ and $Q(0)=\ln(q_0)$. q_0 indicates the physiological state of the cells (Baranyi and Roberts, 1994).

By employing the MATLAB software, the fourth-order Runge-Kutta technique was used to solve these equations. After solving the equations, dynamic time-temperature profiles were used to predict the *B. cereus* population.

Model Validation

Tertiary models were validated by conducting separate experiments using two non-isothermal sinusoidal time-temperature profiles: 5 to 15°C for 27 d and 10 to 40°C for 37 h (Singh et al., 2011). Inoculated meat was prepared, and *B. cereus* population was enumerated as described earlier at pre-determined time points to include the *B. cereus* growth up to the stationary phase.

The accuracy of the predictions of the Baranyi model for each sinusoidal temperature profile was assessed using the acceptable prediction zone (APZ) analysis method (Oscar, 2005). Prediction errors (PE; $\log_{10}$ CFU/g) were calculated for every observation by subtracting the predicted values from the observed values. Fail-dangerous predictions were identified by positive PE values, whereas fail-safe predictions were indicated by negative PE values and a PE of 0 indicated a perfect prediction (Oscar, 2005). The acceptable prediction zone (APZ) limits were established between -1.0 and 0.5 log CFU/g (Mishra et al., 2017).

RESULTS AND DISCUSSION

pH and a_w

The pH values of the prepared turkey breast and RTE turkey breast (before and after heat treatment) were 6.06 ± 0.02 and 6.10 ± 0.011, respectively. The pH values of the turkey breast were in agreement with the literature (Carlson et al., 2005).

Water activity (a_w) of the prepared turkey breast and cooked turkey roast (before and after heat treatment) were 0.981± 0.005 and 0.992 ± 0.010, respectively. The minimum water activity for growth of *B. cereus* was 0.951 and the water activity of the turkey roast will support *B. cereus* growth (Lanciotti et al., 2001). There was no significant difference (P value: <0.05) between before and after heat treatments.

Primary model

The growth data obtained from each isothermal temperature was fitted using the primary Baranyi model (Fig. 3.1). The h_0 values ranged from 1.33 x 10^{-12} to 1.87, with a mean h_0 value of 0.92. The h_0 values can differ by the microorganism and the food matrix. The previous studies have reported different $\overline{h_0}$ values for *Clostridium perfringens* in cooked ham (6.7), *Salmonella* in chicken (1.7), *B. cereus* in cooked rice (4.1), cooked pasta (3.5), and cooked beans (4.8) and *Brochothrix thermosphacta* in a broth media (3.2) (Amezquita et al., 2005; Baranyi and Roberts, 1994; Juneja et al., 2007, 2017; Juneja et al., 2019; Singh et al., 2011). These differences in the h_0 values are probably related to differences in the microorganism(s) and their strain(s), and the growth matrix (food product[s]) and intrinsic and extrinsic properties of the food product used for developing the predictive models. Baranyi model performed well with high R^2 (0.9918) and low RMSE (0.2966) across all growth temperatures (Table – 3.1) (El-Arabi and Griffiths, 2021). In the current study, the lag time specific (λ) at each isothermal temperatures, 15ºC, 20ºC, 25ºC, 30ºC, 35ºC, 40ºC, 45ºC and 50ºC were 16.01, 10.38, 3.95, 2.66, 2.10, 2.05, 2.05 and 24.43 h respectively. The specific growth rate (μ_{max}) and the maximum population densities (y_{max}) for the

respective temperatures were as follows 0.06, 0.09, 0.23, 0.35, 0.44, 0.45, 0.45 and 0.04 log CFU/h and 7.82, 7.99, 7.92, 7.98, 7.92, 7.86, 7.69 and 8.23 log CFU/g respectively (Table-1). All the Baranyi model parameters were in agreement with the earlier literature except for the 50ºC where there was no *B. cereus* growth observed (Juneja et al., 2007, 2017; Juneja et al., 2019).

Insert Figure- 3.1

Secondary model

The kinetic parameters from primary models including lag time and growth rate were integrated into secondary models (Ratkowsky). The Ratkowsky model demonstrated a good fit, with high R^2 and RMSE values of 0.9805 and 0.0260, respectively (Goodness of fit statistics). The estimated T_{min} (5.64°C) and T_{max} (50.32°C) values of Ratkowsky model represent the theoretical (notational) temperatures at which no growth is observed. The observed minimum and maximum growth temperatures (15 and 50°C, respectively; Fig. 3.2) are frequently lower or higher than the extrapolated values (McKellar and Delaquis, 2011). The predicted T_{min} (5.6°C) and T_{max} (50.3°C) values for *B. cereus* growth in RTE turkey breast were similar to those observed in cooked beans and cooked rice (Juneja et al., 2017; Juneja, et al., 2019).

Insert figure - 3.2 and table – 3.1

Dynamic models and Validation:

The tertiary models are applications of secondary models that include algorithms to calculate shifting situations to provide predictions. The primary and secondary models used widely in the food industry and research is combined using these models as computer tools (Ellouze et al., 2021; Psomas and Skandamis, 2019). Dynamic temperature profiles were used for validation of growth model in RTE turkey breast. Inputs for this model were derived from fitted primary and secondary models to predict the growth. From primary growth models parameters like y_0 (2.60),

y_{max} (7.96), and h_0 (0.92) values were used along with the parameters from secondary models like T_{min} (5.64°C), T_{max} (50.32°C), a (0.000611), b (0.1112), c (0.0260) values were used to tertiary models using 4th order Runge-Kutta method (Table 2). The dynamic model was validated using two sinusoidal temperature profiles (5 to 15°C and 10 to 40°C) by comparing the predicted and the observed values (Fig. 3.3). For both the sinusoidal temperature profiles (Figure 3.3), the experimental maximum population density reached the predicted maximum population density, similar to those for *Salmonella* spp. in liquid whole egg (Singh et al., 2011). However, in some instances, this population may not be achieved if adequate time is not allowed for growth, such as in the predictive model for *Listeria monocytogenes* in queso fresco (Thomas et al., 2019). Furthermore, the validity of the growth models can be evaluated using the acceptable prediction zone (APZ) analysis, using prediction errors. For the high-temperature sinusoidal temperature profile, 41 out of 43 observed points and all points (50 out of 50 observations) from low-temperature profile were within APZ limits of the prediction error between -1.0 and 0.5 log CFU/g (Fig. 4). Oscar (2005) stated that an acceptable model would have the prediction errors within 70% (Oscar, 2005). Uneven margins (-1.0 log CFU/g and 0.5 log CFU/g) for the APZ analysis are because prediction error can be tolerated if the prediction error is toward negative direction (Fail-safe). In addition, an excessive fail-safe model leads to the disposal of food which is otherwise safe for consumption, and an excessive fail-dangerous model (>0.5) would lead to the consumption of unsafe foods, thus leading to outbreaks or illnesses. Most of the prediction errors are in fail-safe initially, followed by the opposite trend towards the end of the experiment, indicating overprediction (Fig. 3.4), while the prediction errors were in the fail-dangerous zone, indicating underprediction (Fig. 3.4).

Insert Figure 3.3 and figure 3.4

Conclusion

All goodness of fit parameters were within the acceptable range for Baranyi, Ratkowsky and the dynamic models, indicating that the developed dynamic model performed satisfactorily. The developed dynamic model can be used by the regulatory agencies as well as the food processors to predict potential growth of *B. cereus* in RTE turkey breast under extended storage and temperature abuse conditions to ensure the food safety of the RTE turkey breast. As the dynamic model was developed using conservative formulation (low salt and phosphate content, without any antimicrobial

agents), this model can be widely used by the poultry processors for RTE turkey products as the model would result in a fail-safe prediction. The developed predictive model also helps them in determining the safe cooling rates for cooked meat to prevent the *B. cereus* spore germination and outgrowth.

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References

Amezquita, A., Weller, C. L., Wang, L., Thippareddi, H., & Burson, D. E. (2005). Development of an integrated model for heat transfer and dynamic growth of *Clostridium perfringens* during the cooling of cooked boneless ham. *International Journal of Food Microbiology*, *101*(2), 123–144.

Baranyi, J., & Roberts, T. A. (1994a). A dynamic approach to predicting bacterial growth in food.

International Journal of Food Microbiology, *23*(3–4), 277–294.

Bennett, S. D., Walsh, K. A., & Gould, L. H. (2013). Foodborne disease outbreaks caused by *Bacillus cereus*, *Clostridium perfringens*, and *Staphylococcus aureus*—United States, 1998–2008. *Clinical Infectious Diseases*, *57*(3), 425–433.

Carlin, F., Fricker, M., Pielaat, A., Heisterkamp, S., Shaheen, R., Salonen, M. S., Svensson, B., Nguyen- the, C., & Ehling-Schulz, M. (2006). Emetic toxin-producing strains of *Bacillus cereus* show distinct characteristics

within the *Bacillus cereus* group. *International Journal of Food Microbiology*, *109*(1–2), 132–138.

Carlson, T. R., Marks, B. P., Booren, A. M., Ryser, E. T., & Orta-Ramirez, A. (2005). Effect of water activity on thermal inactivation of *Salmonella* in ground turkey. *Journal of Food Science*, *70*(7), m363–m366.

Chai, T., & Draxler, R. R. (2014). Root mean square error (RMSE) or mean absolute error (MAE)?– Arguments against avoiding RMSE in the literature. *Geoscientific Model Development*, *7*(3), 1247–1250.

Cufaoglu, G., & Ayaz, N. D. (2022). Potential risk of *Bacillus cereus* in spices in Turkey. *Food Control*, *132*, 108570.

Di Bucchianico, A. (2008). Coefficient of determination (R 2). *Encyclopedia of Statistics in Quality and Reliability*.

Eglezos, S., Huang, B., Dykes, G. A., & Fegan, N. (2010). The prevalence and concentration of *Bacillus cereus* in retail food products in Brisbane, Australia. *Foodborne Pathogens and Disease*, *7*(7), 867–870.

El-Arabi, T. F., & Griffiths, M. W. (2021). Bacillus cereus. In *Foodborne infections and intoxications* (pp. 431-437). Academic Press.

Ellouze, M., Buss Da Silva, N., Rouzeau-Szynalski, K., Coisne, L., Cantergiani, F., & Baranyi, J. (2021a). Modeling *Bacillus cereus* growth and cereulide formation in cereal-, dairy-, meat-, vegetable-based food and culture medium. *Frontiers in Microbiology*, *12*, 639546.

Esteban-Cuesta, I., Drees, N., Ulrich, S., Stauch, P., Sperner, B., Schwaiger, K., Gareis, M., & Gottschalk, C. (2018). Endogenous microbial contamination of melons (Cucumis melo) from international trade: an underestimated risk for the consumer? *Journal of the Science of Food and Agriculture*, *98*(13), 5074–5081.

Giannella, R. A., & Brasile, L. (1979). A hospital food-borne outbreak of diarrhea caused by *Bacillus cereus*: clinical, epidemiologic, and microbiologic studies. *Journal of Infectious Diseases*, *139*(3), 366–370.

Granum, P. E. (1994). *Bacillus cereus* and its toxins. *Journal of Applied Microbiology*, *76*(S23), 61S- 66S.

Griffiths, M. W., & Schraft, H. (2017). *Bacillus cereus* food poisoning. In *Foodborne diseases* (pp.395–405). Elsevier.

Hodson, T. O. (2022). Root-mean-square error (RMSE) or mean absolute error (MAE): when to use them or not. *Geoscientific Model Development*, *15*(14), 5481–5487.

Juneja, V. K., Golden, C. E., Mishra, A., Harrison, M. A., & Mohr, T. B. (2019a). Predictive Model for Growth of *Bacillus cereus* at Temperatures Applicable to Cooling of Cooked Pasta. *Journal of Food Science*, *84*(3), 590–598. https://doi.org/10.1111/1750-3841.14448

Juneja, V. K., Golden, C. E., Mishra, A., Harrison, M. A., Mohr, T., & Silverman, M. (2019b). Predictive model for growth of *Bacillus cereus* during cooling of cooked rice. *International Journal of Food Microbiology*, *290*, 49–58. https://doi.org/10.1016/j.ijfoodmicro.2018.09.023

Juneja, V. K., Melendres, M. V., Huang, L., Gumudavelli, V., Subbiah, J., & Thippareddi, H.

(2007a). Modeling the effect of temperature on growth of *Salmonella* in chicken. *Food Microbiology*, *24*(4), 328–335.

Juneja, V. K., Mishra, A., & Pradhan, A. K. (2017a). Dynamic predictive model for growth of *Bacillus cereus* from spores in cooked beans. *Journal of Food Protection*, *81*(2), 308–315.

Juneja, V. K., Osoria, M., Hwang, C.-A., Mishra, A., & Taylor, T. M. (2020). Thermal inactivation of *Bacillus cereus* spores during cooking of rice to ensure later safety of boudin. *LWT*, *122*, 108955. Kang, K.-A., Kim, Y.-W., & Yoon, K.-S. (2010). Development of predictive growth models for Staphylococcus aureus and *Bacillus cereus* on various food matrices consisting of ready-to-eat (RTE) foods. *Food Science of Animal Resources*, *30*(5), 730–738.

Kotiranta, A., Lounatmaa, K., & Haapasalo, M. (2000). Epidemiology and pathogenesis of *Bacillus cereus* infections. *Microbes and Infection*, *2*(2), 189–198.

Koutroumanidis, T., Iliadis, L., & Sylaios, G. K. (2006). Time-series modeling of fishery landings using ARIMA models and Fuzzy Expected Intervals software. *Environmental Modelling & Software*, *21*(12), 1711–1721.

Lanciotti, R., Sinigaglia, M., Gardini, F., Vannini, L., & Guerzoni, M. E. (2001). Growth/no growth interfaces of *Bacillus cereus*, *Staphylococcus aureus* and *Salmonella enteritidis* in model systems based on water activity, pH, temperature and ethanol concentration. *Food Microbiology*, *18*(6), 659–668.

McKellar, R. C., & Delaquis, P. (2011). Development of a dynamic growth–death model for Escherichia coli O157: H7 in minimally processed leafy green vegetables. *International Journal of Food Microbiology*, *151*(1), 7–14.

Messelhäußer, U., & Ehling-Schulz, M. (2018a). *Bacillus cereus*—a multifaceted opportunistic pathogen. *Current Clinical Microbiology Reports*, *5*, 120–125.

Messelhäußer, U., & Ehling-Schulz, M. (2018b). *Bacillus cereus—a multifaceted opportunistic pathogen. Curr Clin Microbiol Rep 5: 120–125*.

Mishra, A., Guo, M., Buchanan, R. L., Schaffner, D. W., & Pradhan, A. K. (2017). Development of growth and survival models for *Salmonella* and *Listeria monocytogenes* during non-isothermal time-temperature profiles in leafy greens. *Food Control, 71*, 32–41.

Oscar, T. E. (2005). Validation of lag time and growth rate models for *Salmonella Typhimurium*: acceptable prediction zone method. *Journal of Food Science*, *70*(2), M129–M137.

Psomas, A., & Skandamis, P. (2019). *GroPIN: Predictive Food Microbial Growth & Inactivation Software (NEW models added: 17-6-2019).*

Ratkowsky, D. A., Lowry, R. K., McMeekin, T. A., Stokes, A. N., & Chandler, R. (1983). Model for bacterial culture growth rate throughout the entire biokinetic temperature range. *Journal of Bacteriology*, *154*(3), 1222–1226.

Ross, T. (1996). Indices for performance evaluation of predictive models in food microbiology. *Journal of Applied Bacteriology*, *81*(5), 501–508.

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V, Widdowson, M.-A., Roy, S. L., Jones, J. L., & Griffin, P. M. (2011). Foodborne illness acquired in the United States—major pathogens. *Emerging Infectious Diseases*, *17*(1), 7–15.

Singh, A., Korasapati, N. R., Juneja, V. K., Subbiah, J., Froning, G., & Thippareddi, H. (2011a). Dynamic predictive model for the growth of *Salmonella* spp. in liquid whole egg. *Journal of Food Science, 76*(3). https://doi.org/10.1111/j.1750-3841.2011.02074.x

Thomas, M., Tiwari, R., & Mishra, A. (2019). Predictive model of *Listeria monocytogenes* growth in queso fresco. *Journal of Food Protection*, *82*(12), 2041–2079. https://doi.org/10.4315/0362- 028X.JFP-19-185

Wijnands, L. M., Dufrenne, J. B., Rombouts, F. M., In't Veld, P. H., & Van Leusden, F. M. (2006). Prevalence of potentially pathogenic *Bacillus cereus* in food commodities in The Netherlands. *Journal of Food Protection*, *69*(11), 2587–2594.

Wijtzes, T., Rombouts, F. M., Kant-Muermans, M. L. T., Van't Riet, K., & Zwietering, M. H. (2001). Development and validation of a combined temperature, water activity, pH model for bacterial growth rate of *Lactobacillus curvatus*. *International Journal of Food Microbiology, 63*(1–2), 57– 64.

Zwietering, M. H., De Koos, J. T., Hasenack, B. E., De Witt, J. C., & Van't Riet, K. (1991). Modeling of bacterial growth as a function of temperature. *Applied and Environmental Microbiology*, *57*(4), 1094–1101.

Table 3.1: Specific growth rate (μmax), lag-time duration (λ), maximum population density (ymax) and goodness of fit parameters such as R2 and RMSE values at each isothermal temperature obtained after fitting B. cereus growth data in RTE turkey breast to the primary Baranyi model.

Temperature (ºC)	μ_{max} (log CFU/h)	λ (h)	y_{max} (log CFU/g)	R^2	RMSE
15	0.06	16.01	7.82	0.9936	0.1702
20	0.09	10.38	7.99	0.9882	0.2307
25	0.23	3.95	7.92	0.9882	0.2384
30	0.35	2.66	7.98	0.9965	0.1286
35	0.44	2.10	7.92	0.9960	0.1456
40	0.45	2.05	7.86	0.9989	0.0704
45	0.45	2.05	7.69	0.9897	0.2195
50	0.04	24.43	8.23	0.9832	1.1696

Table 3.2: Primary (Baranyi) and secondary (modified Ratkowsky) model parameters used to develop dynamic model for growth of *B. cereus* in roast turkey breast.

Models and Parameters	**Values**
Primary Model	
y_0 (log CFU/g)	2.56
y_{max} (log CFU/g)	7.96
h_0	0.92
Secondary Model	
T_{min} (°C)	5.64
T_{max} (°C)	50.32
a	0.0001
b	0.1112

Figure-3.1: Growth of *B. cereus* (Log CFU/g) in RTE Turkey breast at various isothermal temperatures fitted to the primary (Baranyi) model. (Predicted growth: [_____]; Observed growth: Replication-1= [•]; Replication-2= [▪]; and Replication-3=[▲])

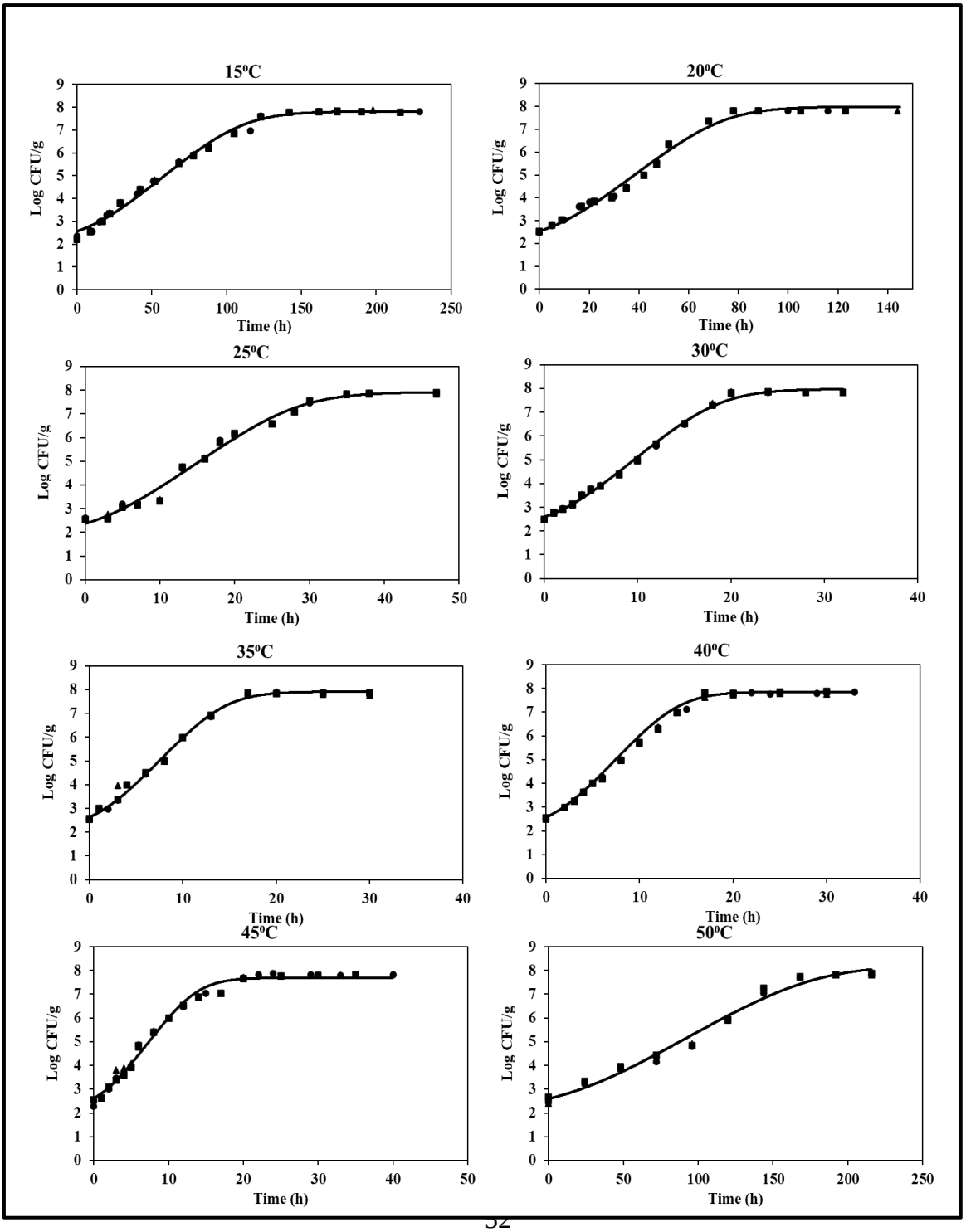


Figure 3.2: Secondary (modified Ratkowsky) model describing the growth rate (log CFU/h) of *B. cereus* in RTE turkey breast as a function of temperature (ºC), compared to growth rates of *B. cereus* rate in roast beef, ham, cooked beans, pasta, and rice.

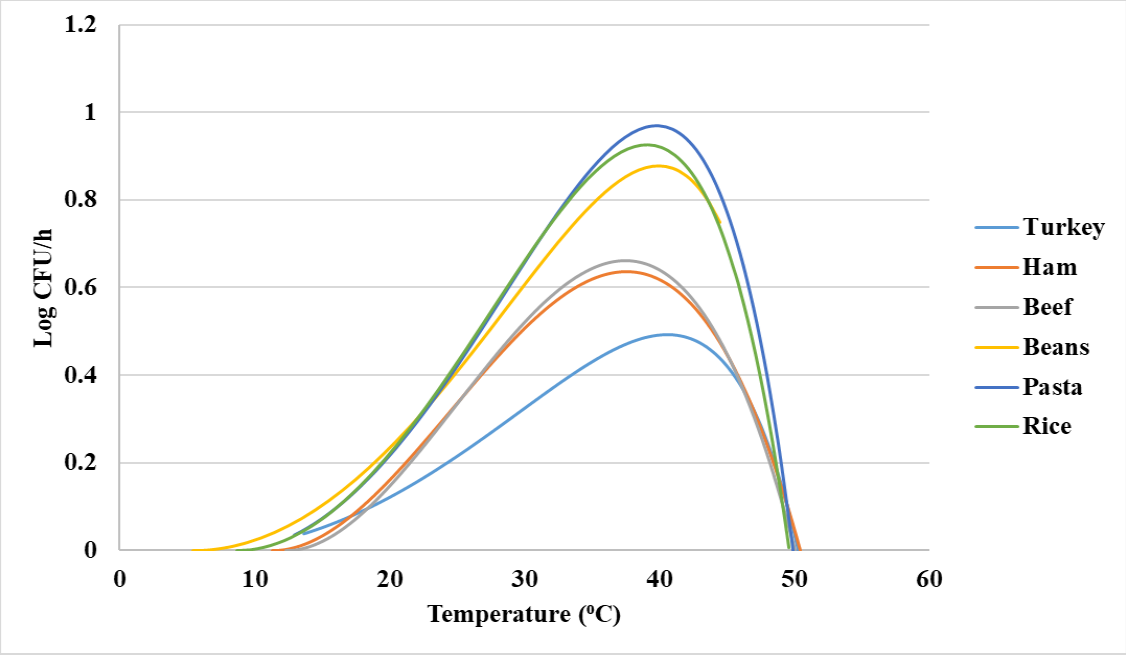


Figure-3.3: Validation of the developed predictive model for *B. cereus* in RTE turkey breast following two sinusoidal temperature profiles (Low temperature profile: 5-15ºC and High temperature profile: 10-40ºC).

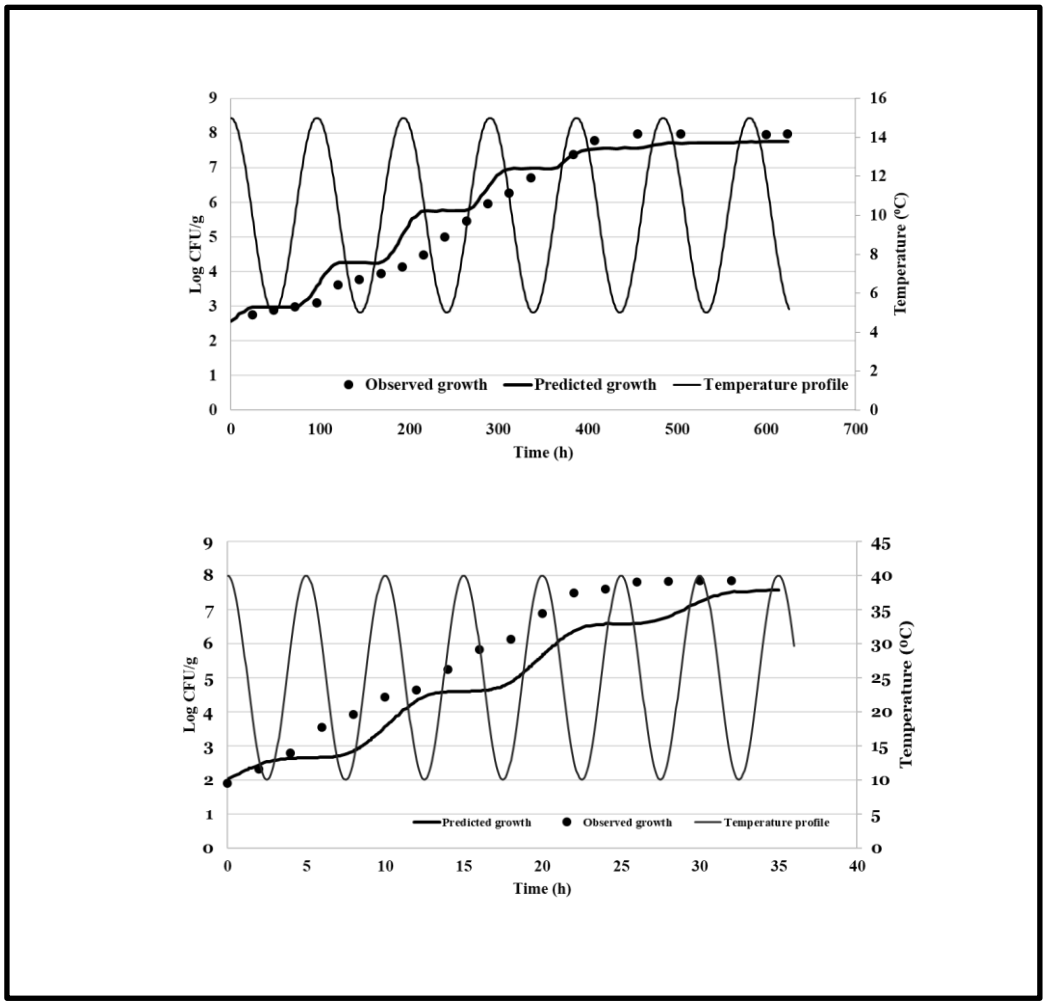
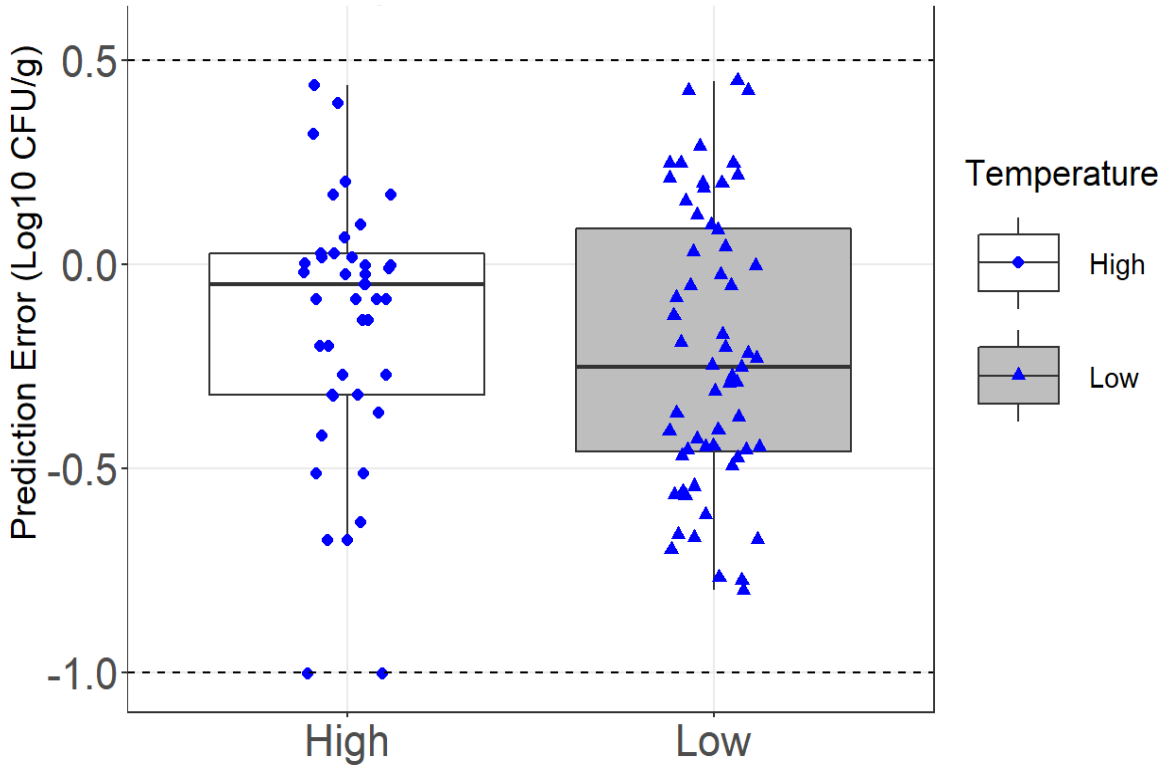


Figure-3.4: Acceptable Prediction Zone (APZ) analysis for the validation experiments - Left boxplot: High-temperature profile: 10-40ºC; Right boxplot: Low-temperature profile: 5-15ºC.



CHAPTER 4

DEVELOPMENT AND VALIDATION OF DYNAMIC PREDICTIVE MODEL FOR THE GROWTH OF BACILLUS CEREUS IN ROAST BEEF

[1]Sujitha Bhumanapalli *et al.* To be submitted to *Journal of Food Protection*

Abstract

Bacillus cereus is a spore producing foodborne pathogen that can cause diarrheal and emetic illnesses in humans. It is commonly found in raw food materials and sometimes in processed foods. Dynamic predictive models for growth of *B. cereus* in meat and poultry products are lacking. A dynamic predictive model for *B. cereus* in roast beef was developed and validated. In this study, ground beef shoulder meat was mixed with a marinade containing 10% water, 1% salt and 0.3% phosphate and inoculated with *B. cereus* spores (~2.5 log CFU/g). The primary Baranyi and the secondary modified Ratkowsky models used to fit the *B. cereus* growth at isothermal temperatures (primary model) and the growth rate at different temperatures (secondary model), respectively. The mean R^2 and RMSE values for the primary model were 0.9860 and 0.2560; and for the secondary model were 0.9795 and 0.0354, respectively. A tertiary dynamic model was developed using the 4^{th}-order Runge-Kutta method and was validated using two sinusoidal temperature profiles (5-15°C and 10-40°C). The accuracy and bias factors for both profiles were within the acceptable range (0.75 – 1.25), and >96% of the predicted errors between predicted and growth observations were within the acceptable prediction zone (-0.5 to 1.0 log CFU/g). The dynamic predictive model can be used to evaluate potential growth of *B. cereus* in roast beef during processing, extended storage, and distribution.

Introduction

Bacillus cereus is ubiquitous gram-positive spore forming foodborne pathogen and is a facultative anaerobe which can grow well under aerobic conditions. The pathogen has been widely associated from the processed beef products such as sausage, minced meat, burger, and luncheon (Juneja et al., 2019). *B. cereus* is an etiological agent of emetic and diarrheal foodborne illnesses (Lee et al., 2006). While some of the pathogenic strains produce heat stable emetic toxin, most of the other

strains produce heat sensitive diarrheal toxin. The emetic toxin causes vomiting within 1 to 6 h of ingestion and diarrheal toxin results in diarrhea within 8 to 24 h of ingestion of contaminated food. The United States Centers for Disease Control and Prevention (CDC) estimated 63,400 cases of foodborne illness and 20 hospitalizations occur annually in the United States due to *B. cereus* (Scallan et al., 2011)

Meat products are generally implicated in most of the *B. cereus* foodborne illness outbreaks (Bennett et al., 2013; DeBuono et al., 1988; Thirkell et al., 2019) The presence of *B. cereus* in commercial, cooked, and pasteurized foods indicates contamination of raw materials with heat resistant spores. *B. cereus* spores may be heat activated during heat treatments such as cooking, subsequently germinating, and outgrowing if improperly cooled, resulting in the production of toxins (Wijnands, 2008). The epidemiological data suggests that the threshold of 5 log CFU/g of *B. cereus* vegetative cells is required to cause both diarrheal and emetic foodborne illnesses in humans (Schraft and Griffiths, 2006). Therefore, food storage at improper temperatures for several hours before serving or inadequate rapid cooling of food after high-temperature cooking could lead to outbreaks in meat products (Adams & Moss, 2000; Mossel et al., 1995).

Several predictive models have been developed for estimating the growth of foodborne pathogens during the cooling of cooked food products. These pathogen growth models are available in ComBase and Pathogen Modeling Program (PMP) by the United States Department of Agriculture, Agricultural Research Service (USDA-ARS). However, those models were developed using microbiological media, under static temperature conditions (Kang et al., 2010). In addition, these models have not been validated for food matrices and do not accurately estimate *B. cereus* growth in cooked beef products. In addition, the available models do not consider the lag phase and growth over the whole biokinetic range, resulting in potentially fail-dangerous predictions.

Currently, there are three dynamic predictive models available for the growth of *B. cereus* spores in cooked beans, rice, and pasta (Juneja et al., 2017; Juneja, Golden, Mishra, Harrison, & Mohr, 2019). The objective of this research was to develop and validate a dynamic predictive model for predicting *B. cereus* growth from spores in roast beef.

The dynamic predictive model developed in this study can be useful for meat processing units as well as regulatory agencies such as USDA-FSIS

to address the public health concern about the *B. cereus* emetic and diarrheal toxins production in roast beef under temperature abuse conditions due to inadequate cooling rate after cooking or improper temperature conditions during storage and supply chain.

METHODS AND MATERIALS

Microorganisms

Four strains of *Bacillus cereus* were obtained from the Center for Food Safety, UGA. All the four strains are diarrheal toxin producing strains which include *B. cereus* F4810/72, isolated from cooked rice, *B. cereus* F4512A/87 isolated from pasteurized milk, *B. cereus* 038-2 isolated from infant formula, and *B. cereus* F3812/84 isolated from pasteurized milk. The individual strains were maintained at -80ºC as stock cultures in cryobeads (Pro-Lab Diagnostics Microbank™, PL.170C/G, Thermo Fisher Scientific, USA).

Preparation of *B. cereus* Spore cocktail

The *B. cereus* spores were prepared using the method described by Juneja *et al.* 2017. Briefly, a bead from each strain was inoculated into sterile brain heart infusion broth (10 mL, BHI) and incubated overnight at 37°C to obtain active vegetative cells at the stationary phase. An aliquot (100 µL) of each active cell suspension was surface plated onto 30 nutrient agar (Difco, BD, Sparks, MD and Fisher Scientific) containing manganese sulfate (0.05 g/L MnSO4; Waltham,

Massachusetts, USA; NAMS). The NAMS plates were incubated for 10 days at 37°C, to yield spores. The plates were flooded with 5 mL of sterile distilled water and the cells were scraped using a sterile plastic spreader. The spore suspension from all 30 NAMS agar plates were collected into conical tubes (50 mL, Falcon® Centrifuge Tube, Conical Bottom, Corning, USA) and centrifuged at 10,000 x *g* for 15 min at 4°C (Centrifuge 5804, Eppendorf, Hamburg, Germany). The supernatant was removed and resuspended the pellet in sterile distilled water (10 mL) and centrifuged as mentioned before. The process was repeated twice before being resuspended the pellet final pellet in sterile distilled water (10 mL) and stored each spore suspension at -20ºC until use. *B. cereus* spore population of each strain was determined by heat treating the spore suspension for 10 min at 80ºC, serially diluted with peptone water (0.1% w/v) and plated on tryptic soya agar (Difco, Detroit, Michigan, USA) containing yeast extract (6g/L; BBL, Franklin Lakes, New Jersey, USA; TSAYE) agar plates. The plates were incubated for 24 h at 37ºC and enumerated. A four-strain *B. cereus* spore cocktail was prepared by combining equal amounts of spores from each strain spore crop (Juneja et al., 2017).

Preparation and inoculation of meat

The beef shoulder was vacuum-packed and shipped under refrigeration from a commercial meat processor. The beef shoulder was cut into 2.5 cm cubes and coarsely ground using 19 mm grinder plate in a meat grinder (PC – 98/32 s/n PC98191917, Mainca USA, ST. Louis, Missouri, USA) and marinated with a marinade of water (10%), salt (1%; Custom blended seasonings, A. C. Legg, Inc., Calera, Alabama, USA) and phosphate (0.3%; Sodium polyphosphate, Brifisol® 512, Ettlinger Corporation, Kansas, USA) by weight of the final product and was mixed for 15 min using a meat mixer (RM -20INT s/n RM20213517, Mainca USA, ST. Louis, Missouri, USA). The marinated meat was then finely ground using 16.35 mm plate (PC – 98/32 s/n

PC98191917, Mainca USA, ST. Louis, Missouri, USA), and vacuum sealed the ground meat (Multivac Sepp Haggenmuller GmbH and Co. KG, Bahnhofstr.4, 87787 Wolferrschwenden, Germany), before being stored at -20ºC until use.

The prepared ground beef meat was thawed overnight was portioned the meat into vacuum bags (5g) bags (Ultravac Chamber Vacuum Packaging Pouches 3 mil, UltraSource LLC, Kansas City, Missouri, USA) and each bag was inoculated with 50 µL of *B. cereus* spore cocktail to achieve ca. 2.5 log CFU/g spore population. Negative controls were prepared by inoculating 50 µl of 0.1% sterile peptone water (PW) without the spore cocktail. The air was removed from the pouches manually excluding the air, heat sealed and thoroughly massaged for 2 min to allow uniform distribution of the spores. The pouches containing the inoculated meat were heat treated for 10 min at 80º in a circulating water bath (MX20H135-A11B, PolyScience, Illinois, USA) and transferred to circulating water baths set at specific temperature (Juneja et al., 2018).

pH and a_w

The pH and water activity (a_w) of the prepared roast beef were determined using a calibrated pH meter (pHTestr® 5F, Oakton, Long Beach, New Jersey, USA) and an a_w meter (Dew Point Water Activity Meter 4TE, AquaLab, Pullman, Washington, USA). Additionally, the pH of heat-treated roast beef (non-inoculated samples) was recorded at four specific sampling points: the initial, the final and two intermediate sampling time intervals, during the storage period (Thomas et al., 2019)

Growth of *B. cereus* at isothermal and non-isothermal profiles

The pouches containing the meat after heat treatment were placed in a circulating water bath (MX20H135-A11B, PolyScience, Illinois, USA) set to constant temperatures between 15°C and 55ºC, covering the *B. cereus* biokinetic growth range. Three independent replications (~15

samples/replication) were performed for each temperature, as represented by meat preparation, spore cocktail and day of inoculation. A sample per replicate was removed at predetermined sampling points and *B. cereus* populations were enumerated, ranging from 32 h and 40 days at 30ºC and 10ºC, respectively.

To validate the developed model, *B. cereus* growth data was collected following two separate time-varying temperature profiles (three independent replicates each). The heat-treated meat pouches were transferred to a programmable chiller (AP15R-40-A11B, Polyscience, Illinois, USA) with continuous water circulation. *B. cereus* growth was determined in the roast beef following two sinusoidal temperature profiles, high temperature (10ºC - 40ºC; 5 h/cycle) and low temperature (10ºC to 15ºC; 6 h/cycle) for 38 h and 26 d, respectively.

Microbial analysis

The meat samples were removed from the water bath at each sampling time and chilled by immersing the bag in an ice water bath for 2 min, and the meat was aseptically transferred to a filter bag (B01385, Whirl-Pak Filter bag, Nasco, Fort Atkinson, Wisconsin, USA). Sterile peptone water (0.1% w/v; 20 mL, PW) was added and the meat was macerated (Basic paddle maxicator, Neutec Group Inc., New York, USA) for 2 min, serially diluted and plated on Aerobic Plate Count Petrifilm™ (3M, St. Paul, MN) (Juneja et al., Golden, Mishra, Harrison, and Mohr, 2019).

Predictive Modeling

Primary growth model

The Baranyi model was used to fit *B. cereus* growth as a function of time at a specific temperature (Baranyi and Roberts, 1994) The model is made up of two differential equations, Equations 1.1 and 1.2.

$$\frac{dy}{dt} = \frac{1}{1+e^{-Q\mu max}}\left(1 - e^{(y-y_{max})}\right) \quad (1.1)$$

$$\frac{dQ}{dt} = \mu_{max} \quad (1.2)$$

The Baranyi model has four parameters to describe bacterial growth under isothermal environmental conditions. This model involves solving the above two equations under specific initial conditions of $y = y_0$ at $t = 0$ and $Q = \log_e(q_0)$, where q indicates the initial physiological state of the microbial cell populations.

$$y^{(t)} = y_0 + \mu_{max} F(t) - \log_e\left(1 + \frac{e^{\mu_{max} F(t)} - 1}{e^{(y_{max} - y_0)}}\right) \quad (2)$$

Where the function

$$F(t) = t + \frac{1}{v}\log_e\left(e^{-vt} + e^{-h_0} - e^{(-vt-h_0)}\right) \quad (3)$$

The parameter y(t), which is the natural logarithm of the concentration, represents the *B. cereus* concentration in CFU/g at a specific time. y_0 and y_{max} are the initial and the maximum populations of *B. cereus* in units of ln CFU/g. The maximum specific growth rate, denoted by μ_{max} (ln CFU/h) is believed to be equal to the limiting rate of increase, ν. The equation $h_0 = \left(\mu_{max}\right) * (\lambda)$, where λ indicates lag time in h and is determined by the below formula:

$$\lambda = \frac{h_0}{\mu_{max}} \quad (4)$$

The growth data was plotted against time, and the values of parameters such as h_0, y_0, y_{max}, and μ_{max} were estimated for each temperature point. The initial values of y_0 and y_{max} were considered as the lower and upper asymptotes, respectively. The maximum growth rate (μ_{max}) was determined from the slope of the exponential phase of bacterial count over time. The initial value of h_0 was calculated by multiplying the lag time (λ) and the maximum growth rate (μ_{max}). The

lag time is the duration between the start of the growth and the start of the exponential growth (Singh et al., 2011).

The *B. cereus* growth data for each temperature was fitted using the curve fitting tool in MATLAB software (Version R2018a, MathWorks, Natick, MA) (Huang, 2013; Mahdinia et al., 2020; Thomas et al., 2019). The parameter h_0 should remain constant when the pre-inoculation conditions of bacterial cells are the same (Baranyi and Roberts, 1994) Therefore, in the initial estimation of the Baranyi model, four parameters (h_0, y_0, y_{max}, and μ_{max}) were determined (Baranyi and Roberts, 1994; Buchanan et al., 1997; Mishra et al., 2017). The mean $h_0$value ($\overline{h_0}$) was calculated from the individual $h_0$value for each temperature, while the other three parameters (y_0, y_{max}, and μ_{max}) were estimated again by fitting them into the Baranyi model using the $\overline{h_0}$ (Huang, 2013; Juneja et al., 2008, 2017).

Secondary Model

The relationship between μ_{max} at each corresponding temperature was determined using secondary model by fitting Baranyi model parameters as a function of temperature (Juneja et al., 2009, 2021). The secondary models consider various biotic and abiotic factors that can influence the kinetics of microbial growth, such as temperature, water activity, pH, and other elements (Baranyi and Roberts, 1994; Gonçalves et al., 2017; Xanthiakos et al., 2006). In this scenario, the only factor that varied was the temperature, while all other extrinsic factors such as water activity, pH and others remained constant, as the same product was used. The Ratkowsky model was utilized as a secondary model to fit the maximum specific growth rates (μ_{max}) and to determine the effect of temperature on μ_{max} (Geeraerd et al., 2004; Ratkowsky et al., 1982, 1983; Ross and Dalgaard, 2004; Zwietering et al., 1991, 1996).

$$\mu_{max} = a(T - T_{min})^2 \{1 - \exp[b(T - T_{max})]\} \quad \text{.....(5)}$$

The equation includes the variables T (temperature in °C), T_{min} and T_{max} (the minimum and maximum theoretical growth limits in °C), and the coefficients a and b, which were determined through regression analysis. Additionally, the lag time at each temperature was modeled using a hyperbolic function. (Zwietering et al., 1991).

$$\lambda = \frac{p}{e^{T-q}} \quad \text{.....(6)}$$

The variable p represents the rate of decrease in lag duration with an increase in temperature while q is the temperature at which the lag phase length becomes infinite, and T is the temperature (ºC). The curve fitting toolbox in MATLAB software (Version R2018a, MathWorks, Natick, MA) was employed to develop secondary models using the trust-region algorithm for non-linear least squares (Juneja, et al. Golden, Mishra, Harrison, and Mohr, 2019; Mahdinia et al., 2020).

Primary and secondary model evaluation - Goodness of fit statistics

The performance of both the primary and secondary models was assessed by using the goodness of fit parameters, accuracy, and bias factors (Ross, 1996). R^2, and root mean square error (RMSE) (Hodson, 2022) To compare the performances of both the models as well as the developed dynamic model (Juneja, Golden, Mishra, Harrison, and Mohr, 2019; Ross, 1996; Hodson, 2022).

The R^2 was calculated using the equation provided below.

$$R^2 = \frac{SSR}{SSTO} \quad \text{.....(7)}$$

where $SSR = \sum_{i=1}^{n} \left(\hat{y}_i - \bar{y}\right)^2$ .....(7.1)

and $SSTO = \sum_{i=1}^{n} \left(y_i - \bar{y}\right)^2$ .....(7.2)

The regression and total sum of squares were represented by SSR and SSTO respectively. The MATLAB was used to compute the goodness of fit statistics.

The following equation was used to calculate the RMSE value (Chai and Draxler, 2014).

$$\sqrt{\frac{1}{n}\sum_{i=1}^{n} e_i^2} \quad \text{.....(8)}$$

The variables in the formula are as follows: n is the sample size, $\hat{y}$ represents the predicted regression, $\overline{y}$ is the mean response variable, y is response variable (*B. cereus* population density)

$$\text{Accuracy factor} = 10^{\frac{\sum\left|\log\left(\frac{GT_{predicted}}{GT_{observed}}\right)\right|}{n}} \quad \text{.....(9)}$$

$$\text{Bias factor} = 10^{\frac{\sum \log\left(\frac{GT_{predicted}}{GT_{observed}}\right)}{n}} \quad \text{.....(10)}$$

The variable $GT_{predicted}$ represents the predicted generation time (in hours), $GT_{observed}$ represents the observed generation time (in hours) and n represents the sample size.

Tertiary (dynamic) Model

Tertiary models were developed by integrating primary and secondary models using MATLAB software (Butcher, 1996; Velugoti et al., 2011). Baranyi and Roberts (1994) described the differential growth in the model by using two first – order differential equations (Baranyi and Roberts, 1994).

$$\frac{dy}{dt} = \frac{1}{1+e^{-Q(t)}}\mu_{max}[T(t)]\left(1-e^{y(t)-y_{max}}\right) \quad \text{.....(11)}$$

$$\frac{dQ}{dt} = \mu_{max}[T(t)] \quad \text{.....(12)}$$

where $Q(t)=\ln q(t)$

The initial conditions for the model are indicated by y_0 and Q (0), y_0 denotes the initial population density of *B. cereus* and Q(0) represents the initial physiological state of the cells. The value of Q(0) is calculated as the natural logarithm of q_0. The differential equations for the model were solved using the fourth order Runge-Kutta method in MATLAB software (Butcher, 1996). The dynamic time-temperature profiles were then used to predict the *B. cereus* population density (Gumudavelli et al., 2007; Velugoti et al., 2011).

Model Validation

Two separate experiments were conducted to validate the tertiary models using dynamic time-temperature profiles: one ranging from 5 to 15°C for 26 days and the other ranging from 10 to 40°C for 38 hours (Singh et al., 2011). The meat samples have been collected and enumerated the *B. cereus* population at predetermined sampling points, which includes the *B. cereus* growth till the stationary phase.

The accuracy of the Baranyi model's predictions for each dynamic temperature profile was evaluated using acceptable prediction zone (APZ) analysis (T. E. Oscar, 2005; T. P. Oscar, 2005; Wang et al., 2017). Prediction errors (PE; $\log_{10}$ CFU/g) were calculated for each observation by subtracting the predicted values from the observed values. Fail-dangerous predictions were identified by positive PE values, while fail-safe predictions were indicated by negative PE values; a PE of 0 meant a perfect prediction (T. E. Oscar, 2005; T. P. Oscar, 2005). The acceptable prediction zone (APZ) limits were set between -1.0 to 0.5 log CFU/g (Juneja et al., 2017; Thomas et al., 2019).

RESULTS AND DISCUSSION

pH and a_w

The pH of the prepared beef shoulder and cooked beef roast (before and after heat treatment) were 5.98 ± 0.01 and 6.03 ± 0.02, respectively. The pH values of the prepared roast beef were similar to the reported values in literature (Neath et al., 2007).

The water activity (a_w) measurements for the prepared beef shoulder and cooked beef roast (before and after heat treatment) were determined as 0.988 ± 0.004 and 0.986 ± 0.002, respectively. It is important to note that the minimum water activity required for the growth of *B. cereus* was found to be 0.951, indicating that the water activity of the beef roast can support the growth of *B. cereus* (Lanciotti et al., 2001). No significant differences were observed between the water activity values of the prepared raw beef and the roast beef were similar ($P < 0.05$).

Primary model

The growth data of *B. cereus* determined at various isothermal temperatures were fitted to primary Baranyi model (Fig. 4.1). In this study, the h_0 values ranged from 9.11 x 10^{-10} to 4.16, with a mean value of 1.49. The mean h_0 value was found to be different among various pathogens in various food matrices. For example, in cooked ham, *Clostridium perfringens* has an h_0 value of 6.7, while *Salmonella* in chicken has an h_0 value of 1.7. *Bacillus cereus* has an h_0 value of 4.1 in cooked rice, 3.46 in cooked pasta, and 4.80 in cooked beans (Amezquita et al., 2005; Juneja et al., 2007, 2017; Juneja, Golden, Mishra, Harrison, Mohr, et al., 2019). *Brochothrix thermosphacta* has an h_0 value of 3.2 in broth medium (Baranyi et al., 1995). The disparities in the average h_0 values can be attributed to variations in the microorganisms and their strains, as well as the composition and intrinsic and extrinsic properties of the food product(s) used to develop the predictive models.

The Baranyi model performed well with high R^2 (0.9860) and low RMSE (0.2560) across all growth temperatures, indicating its predictability for *B. cereus* growth in cooked beef roast (El-

Arabi and Griffiths, 2021; Juneja et al., 2008; Juneja et al., Golden, Mishra, Harrison, and Mohr, 2019; Singh et al., 2011). In the current study, specific growth rates (μ_{max}) at each isothermal temperatures like 15ºC, 20ºC, 25ºC, 30ºC, 35ºC, 40ºC, 45ºC and 50ºC were as follows 0.07, 0.10, 0.38, 0.50, 0.67, 0.46, 0.53 and 0.04 log CFU/h respectively. Furthermore, the lag time durations (λ) for the respective isothermal temperatures are 21.16, 15.26, 3.94, 2.96, 2.42, 2.48, 3.21 and 34 h. Furthermore, at these temperatures the maximum population densities (y_{max}) were 7.84, 8.16, 7.82, 7.87, 7.84, 7.72, 7.57 and 7.84 log CFU/g respectively. The goodness of fit parameters like R^2 and RMSE values were represented below (Table – 4.1). Previous studies also stated similar Baranyi model parameter values except that they did not observe *B. cereus* growth at 50ºC like in this study (Juneja et al., 2008; Juneja et al. 2019).

Secondary model

The study incorporated kinetic parameters lag phase duration and growth rate from primary models into the Ratkowsky model (secondary model), which fit the data well with the observed maximum specific growth rate of *B. cereus* in roast beef (Fig. 4.2). The estimated T_{min} (11.37ºC) and T_{max} (50.44ºC) values from the Ratkowsky model represent the theoretical temperatures at which no growth is observed and agreed with the observed T_{min} (10ºC) and T_{max} (53ºC) values (McKellar and Delaquis, 2011). The modified Ratkowsky model was found suitable to estimate the maximum specific growth rate of *B. cereus* in roast beef based on the goodness of fit statistics, where the model was fitted well to observed μ_{max} values for each primary model. This is supported by the high R^2 value of 0.9795 and the low RMSE value of 0.0354 $\log_{10}$ CFU/g. The predicted T_{min} and T_{max} values for roast beef in this study were like those predicted for cooked pasta (8.99 and 49.92ºC, respectively) and cooked rice (8,69 and 49.68ºC, respectively) (Juneja, Golden, Mishra, Harrison, Mohr, et al., 2019). Additionally, the effect of temperature on the lag

phase duration of *B. cereus* in roast beef was described using a hyperbolic function, which fit well with the Baranyi model. The estimated parameters for the primary models are presented in Table 4.1. According to the secondary Ratkowsky model, the growth rate increased with an increase in temperature till 35ºC and then decreased from 40ºC to 50ºC (figure – 4.2). The maximum growth rate observed at 35°C was 0.62 log CFU/h, which was in accordance with the highest growth rate of *B. cereus* in cooked beans (0.6 log CFU/h). The lowest growth rate observed at 50ºC was 0.00439 log CFU/h. In contrast, the lowest growth rate was at 13ºC, which was 0.16 log CFU/h (Juneja et al., 2017).

Insert Fig. 4.2 and Table 4.1.

Tertiary (dynamic) model and Validation

The tertiary models combine primary and secondary models to provide microbial growth predictions by applying algorithms to calculate shifting environmental conditions such as temperature (Ellouze et al., 2021b; Psomas & Skandamis, 2019). Validation of growth models in roast beef was performed using dynamic temperature profiles (5 - 15ºC and 10 - 40ºC) (Fig. 4.3). Inputs for this model were obtained from fitted primary and secondary models to predict the growth. The primary growth model parameters y_0 (2.53), y_{max} (7.75), and h_0 (1.49), as well as the parameters from the secondary models T_{min} (11.37ºC), T_{max} (50.44ºC), a (0.0269) and b (0.0027), were used as inputs in the tertiary model using the 4^{th} order Runge-Kutta method (Table-4.2).

The dynamic model was validated (Fig. 3) following two sinusoidal temperature profiles (5 to15ºC and 10 to 40ºC) and the observed growth was compared with the predicted growth. For both the profiles, the experimental maximum population density reached the predicted maximum population density, as reported for *Salmonella* spp. in liquid whole eggs (Singh et al., 2011;

Thomas et al., 2019). The dynamic model performance was evaluated using the acceptable prediction zone (APZ) analysis method and the prediction errors (PE) indicated that the dynamic model performed well.

The APZ analysis indicated that 48 out of 51 observed points from the high-temperature profile and 42 out of 42 points from the low-temperature profile were within the APZ limits (prediction error between -1.0 and 0.5 log CFU/g; Fig. 4.4). A model is usually considered acceptable if the PE is within 70%. Uneven margins for the APZ analysis are used as the prediction error (log CFU/g) can be tolerated if the prediction errors are towards the negative direction (fail-safe; Fig. 4.4). Furthermore, an excessive fail-safe model leads to the disposal of food that is safe for consumption, and an excessive fail-dangerous model (PE beyond 0.5 log CFU/g) would lead to the consumption of unsafe foods, leading to outbreaks or illnesses. Most of the predictions were in agreement with the observed growth (Fig. 4.4), except from 180 to 350 h and from 14 to 18 h in low and high sinusoidal temperature profiles, respectively. The PEs at these time points were marginally (average PE values: -0.66 and -0.34 respectively) towards negative side (fail-safe), which is like the predictions observed by Singh et al., 2011.

Insert Figs. 4.3 and 4.4.

Conclusion

The developed dynamic model using the Baranyi and the Ratkowsky as primary and secondary models showed good performance, with all goodness of fit parameters within acceptable ranges. Regulatory agencies and food processors can use these models to predict the growth of *B. cereus* in ready-to-eat (RTE) roast beef under different storage and temperature conditions to assure food safety. These models also help in determining safe cooling rates for cooked RTE meats like roast beef to minimize the risk of *B. cereus* spore germination and outgrowth.

Acknowledgment

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References

Adams, M. R., & Moss, M. O. (2000). *Bacillus cereus* and other *Bacillus* species. *Food Microbiology. MPG Books Ltd., Bodmin, UK*, 187–192.

Amezquita, A., Weller, C. L., Wang, L., Thippareddi, H., & Burson, D. E. (2005). Development of an integrated model for heat transfer and dynamic growth of *Clostridium perfringens* during the cooling of cooked boneless ham. *International Journal of Food Microbiology*, *101*(2), 123–144.

Baranyi, J., & Roberts, T. A. (1994). A dynamic approach to predicting bacterial growth in food. *International Journal of Food Microbiology*, *23*(3–4), 277–294.

Baranyi, J., Robinson, T. P., Kaloti, A., & Mackey, B. M. (1995). Predicting growth of *Brochothrix thermosphacta* at changing temperature. *International Journal of Food Microbiology*, *27*(1), 61–75.

Bennett, S. D., Walsh, K. A., & Gould, L. H. (2013). Foodborne disease outbreaks caused by *Bacillus cereus*, *Clostridium perfringens*, and *Staphylococcus aureus*—United States, 1998–2008. *Clinical Infectious Diseases*, *57*(3), 425–433.

Buchanan, R. L., Whiting, R. C., & Damert, W. C. (1997). When is simple good enough: a comparison of the Gompertz, Baranyi, and three-phase linear models for fitting bacterial growth curves. *Food Microbiology*, *14*(4), 313–326.

Butcher, J. C. (1996). A history of Runge-Kutta methods. *Applied Numerical Mathematics*, *20*(3), 247–260.

Chai, T., & Draxler, R. R. (2014). Root mean square error (RMSE) or mean absolute error (MAE)?–Arguments against avoiding RMSE in the literature. *Geoscientific Model Development*, *7*(3), 1247–1250.

DeBuono, B. A., Brondum, J., Kramer, J. M., Gilbert, R. J., & Opal, S. M. (1988). Plasmid, serotypic, and enterotoxin analysis of *Bacillus cereus* in an outbreak setting. *Journal of Clinical Microbiology*, *26*(8), 1571–1574.

El-Arabi, T. F., & Griffiths, M. W. (2021). *Baci*llus cereus. In *Foodborne infections and intoxications* (pp. 431-437). Academic Press.

Ellouze, M., Buss Da Silva, N., Rouzeau-Szynalski, K., Coisne, L., Cantergiani, F., & Baranyi, J. (2021). Modeling *Bacillus cereus* growth and cereulide formation in cereal-, dairy-, meat-, vegetable-based food and culture medium. *Frontiers in Microbiology*, *12*, 639546.

Geeraerd, A. H., Valdramidis, V. P., Devlieghere, F., Bernaert, H., Debevere, J., & Van Impe, J. F. (2004). Development of a novel approach for secondary modelling in predictive microbiology: incorporation of microbiological knowledge in black box polynomial modelling. *International Journal of Food Microbiology*, *91*(3), 229–244.

Gonçalves, L. D. dos A., Piccoli, R. H., & Peres, A. de P. (2017). Predictive modeling of *Pseudomonas fluorescens* growth under different temperature and pH values. *Brazilian Journal of Microbiology*, *48*, 352–358.

Granum, P. E. (1994). *Bacillus cereus* and its toxins. *Journal of Applied Microbiology*, *76*(S23), 61S-66S.

Gumudavelli, V., Subbiah, J., Thippareddi, H., Velugoti, P. R., & Froning, G. (2007). Dynamic predictive model for growth of *Salmonella enteritidis* in egg yolk. *Journal of Food Science*, *72*(7). https://doi.org/10.1111/j.1750-3841.2007.00444.x

Hodson, T. O. (2022). Root-mean-square error (RMSE) or mean absolute error (MAE): when to use them or not. *Geoscientific Model Development*, *15*(14), 5481–5487.

Huang, L. (2013a). Optimization of a new mathematical model for bacterial growth. *Food Control*, *32*(1), 283–288.

Huang, L. (2013b). Optimization of a new mathematical model for bacterial growth. *Food Control*, *32*(1), 283–288.

Juneja, V. K., Golden, C. E., Mishra, A., Harrison, M. A., & Mohr, T. B. (2019). Predictive model for growth of *Bacillus cereus* at temperatures applicable to cooling of cooked pasta. *Journal of Food Science*, *84*(3), 590–598.

Juneja, V. K., Golden, C. E., Mishra, A., Harrison, M. A., Mohr, T., & Silverman, M. (2019). Predictive model for growth of *Bacillus cereus* during cooling of cooked rice. *International Journal of Food Microbiology*, *290*, 49–58.

Juneja, V. K., Marks, H., & Thippareddi, H. (2008). Predictive model for growth of *Clostridium perfringens* during cooling of cooked uncured beef. *Food Microbiology*, *25*(1), 42–55. https://doi.org/10.1016/j.fm.2007.08.004

Juneja, V. K., Melendres, M. V., Huang, L., Gumudavelli, V., Subbiah, J., & Thippareddi, H. (2007). Modeling the effect of temperature on growth of *Salmonella* in chicken. *Food Microbiology*, *24*(4), 328–335.

Juneja, V. K., Melendres, M. V., Huang, L., Subbiah, J., & Thippareddi, H. (2009). Mathematical modeling of growth of Salmonella in raw ground beef under isothermal conditions from 10 to 45 C. *International Journal of Food Microbiology*, *131*(2–3), 106–111.

Juneja, V. K., Mishra, A., & Pradhan, A. K. (2017). Dynamic predictive model for growth of *Bacillus cereus* from spores in cooked beans. *Journal of Food Protection*, *81*(2), 308–315.

Juneja, V. K., Purohit, A. S., Golden, M., Osoria, M., Glass, K. A., Mishra, A., Thippareddi, H., Devkumar, G., Mohr, T. B., Minocha, U., Silverman, M., & Schaffner, D. W. (2021). A predictive growth model for *Clostridium botulinum* during cooling of cooked uncured ground beef. *Food Microbiology*, *93*. https://doi.org/10.1016/j.fm.2020.103618

Kang, K.-A., Kim, Y.-W., & Yoon, K.-S. (2010). Development of predictive growth models for *Staphylococcus aureus* and *Bacillus cereus* on various food matrices consisting of ready-to-eat (RTE) foods. *Food Science of Animal Resources*, *30*(5), 730–738.

Lanciotti, R., Sinigaglia, M., Gardini, F., Vannini, L., & Guerzoni, M. E. (2001). Growth/no growth interfaces of *Bacillus cereus*, *Staphylococcus aureus* and *Salmonella enteritidis* in model systems based on water activity, pH, temperature, and ethanol concentration. *Food Microbiology*, *18*(6), 659–668.

Mahdinia, E., Liu, S., Demirci, A., & Puri, V. M. (2020). Microbial growth models. *Food Safety Engineering*, 357–398.

McKellar, R. C., & Delaquis, P. (2011). Development of a dynamic growth–death model for *Escherichia coli* O157: H7 in minimally processed leafy green vegetables. *International Journal of Food Microbiology*, *151*(1), 7–14.

Mishra, A., Guo, M., Buchanan, R. L., Schaffner, D. W., & Pradhan, A. K. (2017). Development of growth and survival models for *Salmonella* and *Listeria monocytogenes* during non-isothermal time-temperature profiles in leafy greens. *Food Control*, *71*, 32–41.

Mossel, D. A. A., Corry, J. E. L., Struijk, C. B., & Baird, R. M. (1995). *Essentials of the microbiology of foods: a textbook for advanced studies.* John Wiley & Sons.

Neath, K. E., Del Barrio, A. N., Lapitan, R. M., Herrera, J. R. V, Cruz, L. C., Fujihara, T., Muroya, S., Chikuni, K., Hirabayashi, M., & Kanai, Y. (2007). Difference in tenderness and pH

decline between water buffalo meat and beef during postmortem aging. *Meat Science*, *75*(3), 499–505.

Oscar, T. E. (2005). Validation of lag time and growth rate models for *Salmonella Typhimurium*: acceptable prediction zone method. *Journal of Food Science*, *70*(2), M129–M137.

Oscar, T. P. (2005). Development and validation of primary, secondary, and tertiary models for growth of *Salmonella Typhimurium* on sterile chicken. *Journal of Food Protection*, *68*(12), 2606–2613.

Psomas, A., & Skandamis, P. (2019). *GroPIN: Predictive Food Microbial Growth & Inactivation Software (NEW models added: 17-6-2019).*

Ratkowsky, D. A., Lowry, R. K., McMeekin, T. A., Stokes, A. N., & Chandler, R. (1983). Model for bacterial culture growth rate throughout the entire biokinetic temperature range. *Journal of Bacteriology*, *154*(3), 1222–1226.

Ratkowsky, D. A., Olley, J., McMeekin, T. A., & Ball, A. (1982). Relationship between temperature and growth rate of bacterial cultures. *Journal of Bacteriology*, *149*(1), 1–5.

Ross, T. (1996). Indices for performance evaluation of predictive models in food microbiology. *Journal of Applied Bacteriology*, *81*(5), 501–508. https://doi.org/10.1111/j.1365-2672.1996.tb03539.x

Ross, T., & Dalgaard, P. (2004). Secondary models. *Modeling Microbial Responses in Food*, *17*, 360.

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V, Widdowson, M.-A., Roy, S. L., Jones, J. L., & Griffin, P. M. (2011). Foodborne illness acquired in the United States—major *pathogens*. *Emerg Infect Dis*, *17*(1), 7–15.

Shawish, R., & Tarabees, R. (2017). Prevalence and antimicrobial resistance of *Bacillus cereus*

isolated from beef products in Egypt. *Open Veterinary Journal*, *7*(4), 337–341.

Singh, A., Korasapati, N. R., Juneja, V. K., Subbiah, J., Froning, G., & Thippareddi, H. (2011). Dynamic predictive model for the growth of *Salmonella* spp. in liquid whole egg. *Journal of Food Science*, *76*(3), M225–M232.

Thirkell, C., Sloan-Gardner, T., Kaczmarek, M., & Polkinghorne, B. (2019). *An outbreak of Bacillus cereus toxin-mediated emetic and diarrhoeal syndromes at a restaurant in Canberra, Australia 2018*.

Thomas, M., Tiwari, R., & Mishra, A. (2019). Predictive model of *Listeria monocytogenes* growth in queso fresco. *Journal of Food Protection*, *82*(12), 2041–2079. https://doi.org/10.4315/0362-028X.JFP-19-185

Velugoti, P. R., Bohra, L. K., Juneja, V. K., Huang, L., Wesseling, A. L., Subbiah, J., & Thippareddi, H. (2011a). Dynamic model for predicting growth of *Salmonella spp*. in ground sterile pork. *Food Microbiology*, *28*(4), 796–803.

Velugoti, P. R., Bohra, L. K., Juneja, V. K., Huang, L., Wesseling, A. L., Subbiah, J., & Thippareddi, H. (2011b). Dynamic model for predicting growth of *Salmonella* spp. in ground sterile pork. *Food Microbiology*, *28*(4), 796–803.

Wang, J., Koseki, S., Chung, M.-J., & Oh, D.-H. (2017). A novel approach to predict the growth of *Staphylococcus aureus* on rice cake. *Frontiers in Microbiology*, *8*, 1140.

Wijnands, L. M. (2008). *Bacillus cereus* associated food borne disease quantitative aspects of exposure assessment and hazard characterization. Wageningen University and Research.

Xanthiakos, K., Simos, D., Angelidis, A. S., Nychas, G. J. E., & Koutsoumanis, K. (2006). Dynamic modeling of *Listeria monocytogenes* growth in pasteurized milk. *Journal of Applied Microbiology*, *100*(6), 1289–1298. https://doi.org/10.1111/j.1365-2672.2006.02854.x

Zwietering, M. H., De Koos, J. T., Hasenack, B. E., De Witt, J. C., & Van't Riet, K. (1991). Modeling of bacterial growth as a function of temperature. *Applied and Environmental Microbiology*, *57*(4), 1094–1101.

Zwietering, M. H., De Wit, J. C., & Notermans, S. (1996). Application of predictive microbiology to estimate the number of *Bacillus cereus* in pasteurised milk at the point of consumption. *International Journal of Food Microbiology*, *30*(1–2), 55–70.

Table 4.1: Specific growth rate (μmax), lag-time duration (λ), maximum population density (ymax) and goodness of fit parameters such as R2 and RMSE values at each isothermal temperature obtained after fitting B. cereus growth data in roast beef to the primary (Baranyi) model.

Temperature	μ_{max} (Log CFU/h)	λ (h)	y_{max} (Log CFU/g)	R^2	RMSE
15	0.07	2.69	7.84	0.9864	0.2531
20	0.10	2.66	8.16	0.9891	0.2427
25	0.38	2.50	7.82	0.9862	0.2687
30	0.50	2.57	7.87	0.9878	0.2465
35	0.67	2.23	7.84	0.9833	0.2881
40	0.46	2.58	7.72	0.9832	0.2883
45	0.53	2.48	7.57	0.9813	0.3132
50	0.04	2.57	7.84	0.9846	0.2822

Table 4.2: Primary (Baranyi) and secondary (modified Ratkowsky) model parameters used to develop dynamic model for growth of *B. cereus* in roast beef.

Models and Parameters	**Values**
Primary Model	
y_0 (log CFU/g)	2.53
y_{max} (log CFU/g)	7.75
h_0	1.49
Secondary Model	
T_{min} (°C)	11.37
T_{max} (°C)	50.44
a	0.0269
b	0.0027

Figure-4.1: Growth of *B. cereus* (log CFU/g) in roast beef at various isothermal temperatures fitted to the Primary (Baranyi) model.

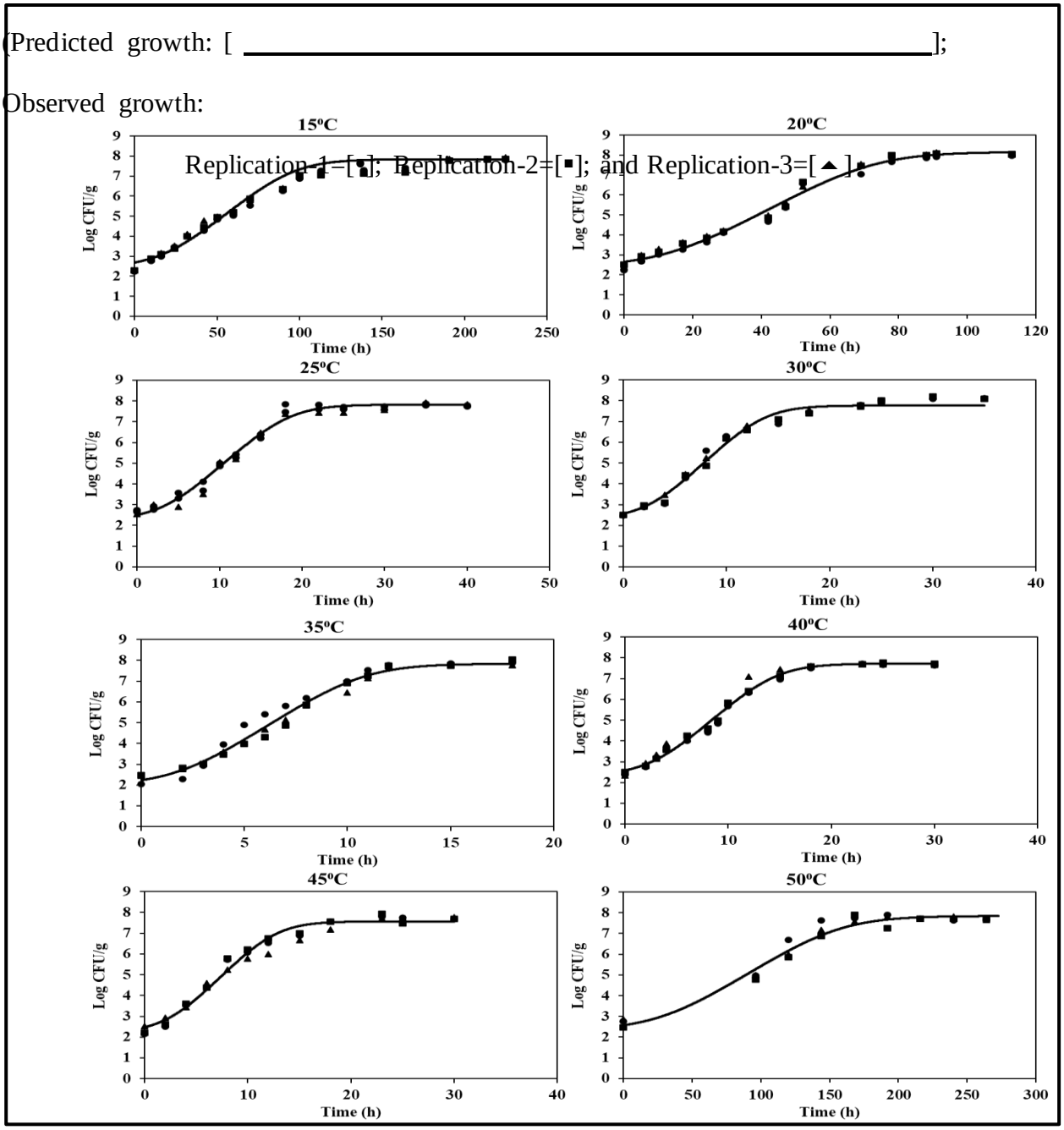


Figure 4.2: Secondary (modified Ratkowsky) model describing the growth rate (log CFU/h) of B. cereus in roast beef as a function of temperature (ºC), compared to growth rate of B. cereus rates in RTE turkey breast, ham, cooked beans, pasta, and rice.

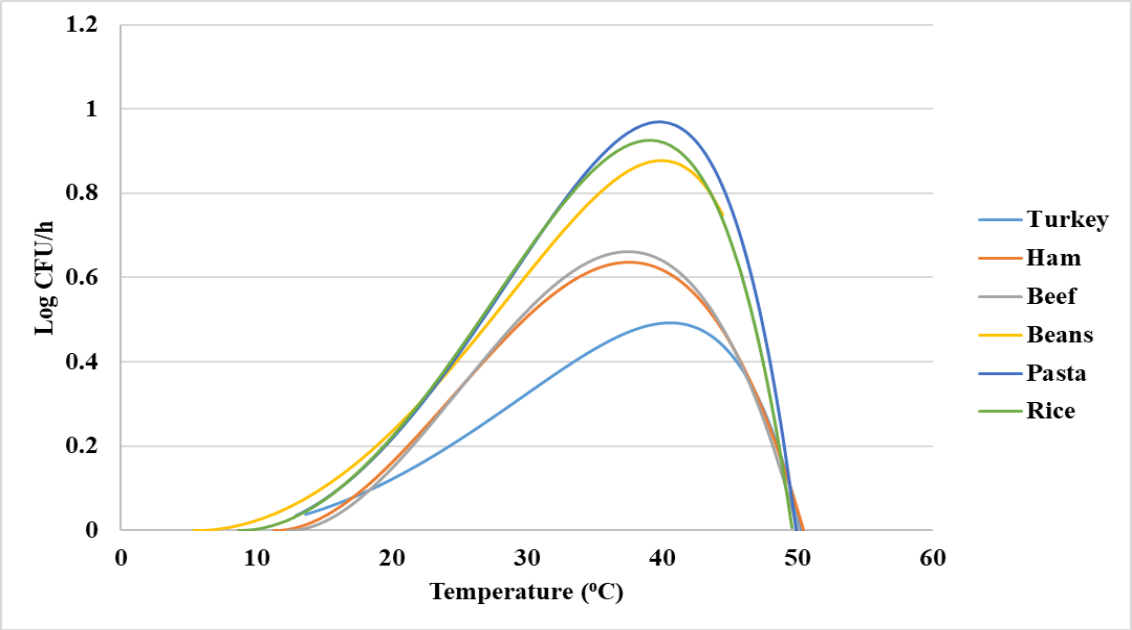


Figure 4.3: Validation of the developed predictive model for *B. cereus* in roast beef following two sinusoidal temperature profiles (Low temperature profile: 5-15ºC and High temperature profile: 10-40ºC).

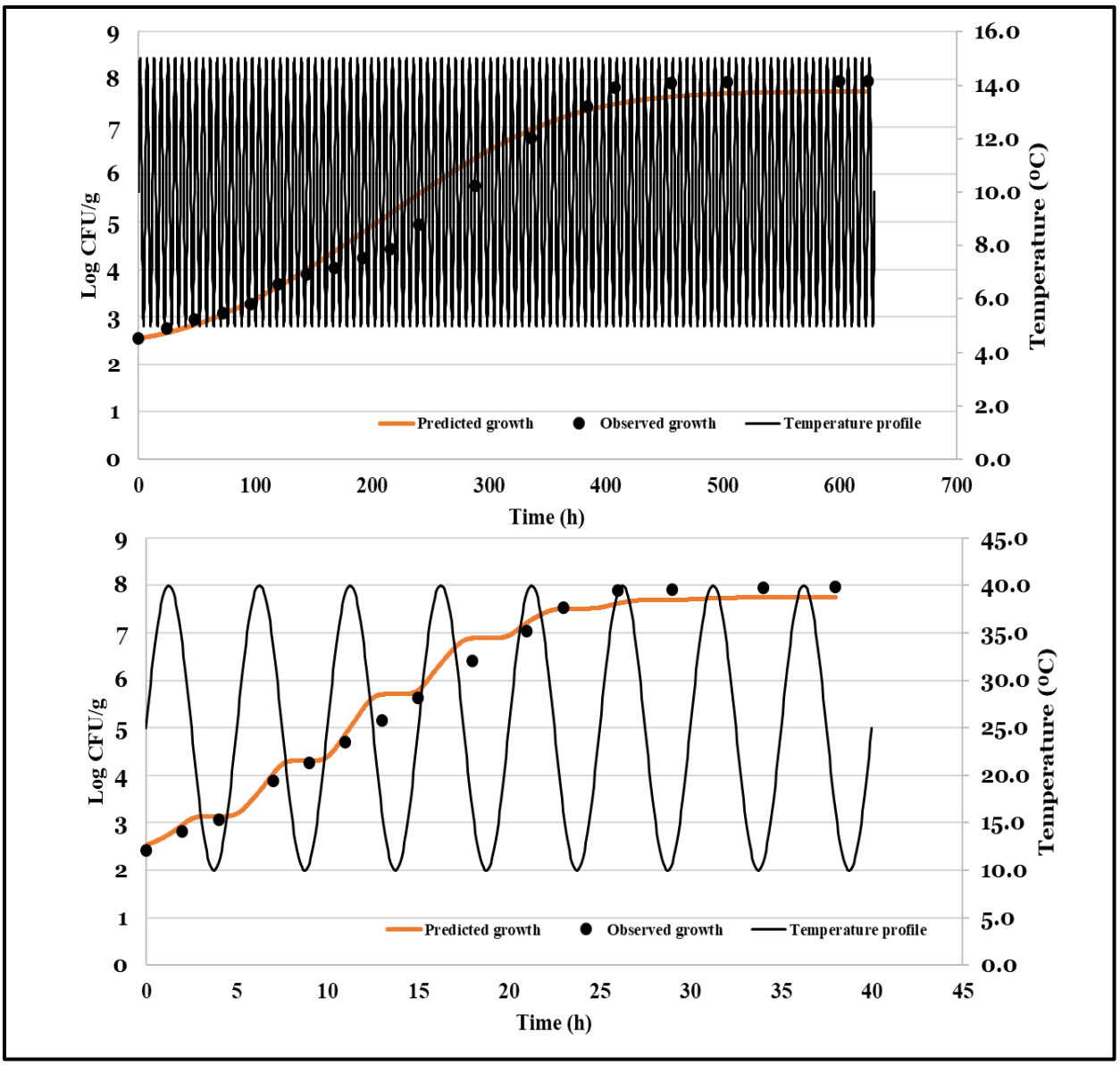
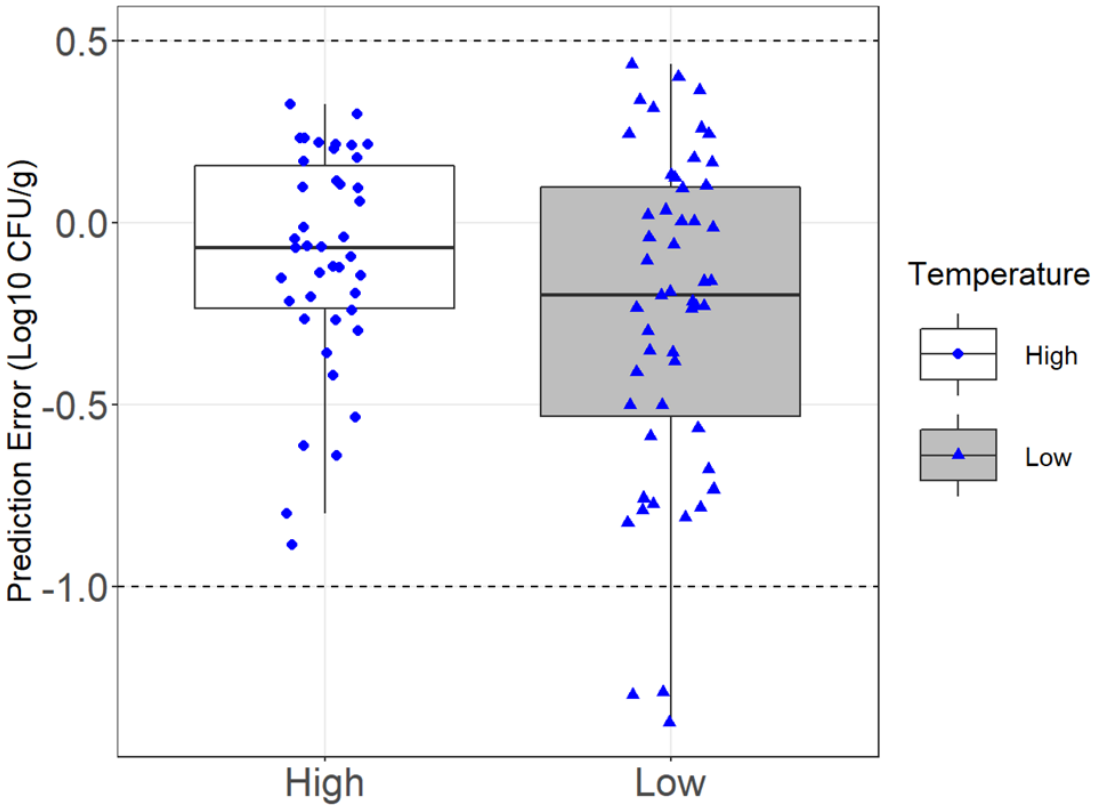


Figure 4.4: Acceptable Prediction Zone (APZ) analysis for the validation experiments - Left boxplot: High temperature profile: 10-40ºC; Right boxplot: Low temperature profile: 5-15ºC.



CHAPTER 5

DEVELOPMENT AND VALIDATION OF DYNAMIC PREDICTIVE MODEL FOR THE GROWTH FOR BACILLUS CEREUS IN HAM

[1]Sujitha Bhumanapalli *et al.* To be submitted to *Journal of Food Protection*

Abstract

B. cereus is ubiquitous foodborne pathogen that produces spores and can cause emetic and diarrheal syndromes in humans. The pathogen is a common food contaminant, typically present in uncooked food products as well as some processed foods. At the moment, there are no dynamic predictive models available to predict the growth of *B. cereus* in ham. This study of developing and validating of dynamic predictive model for the growth of *B. cereus* in ham fills the gap in knowledge. The ground ham meat was mixed with a marinade (10% water, 1% salt, and 0.3% phosphate) and was inoculated with *B. cereus* spores (approximately 2.5 log10 CFU/g). The growth data of *B. cereus* at different isothermal temperatures were collected, and the Baranyi model was used as a primary model to fit the growth data. A modified Ratkowsky model was used as a secondary model to fit the parameters obtained from the Baranyi model as a function of temperature. The primary model had mean R^2 and RMSE values of 0.9873 and 0.2392 log10 CFU/g, respectively, while the secondary model had mean R^2 and RMSE values of 0.9435 and 0.0537 log10 CFU/g, respectively. Using the 4th-order Runge-Kutta method, a tertiary dynamic model was developed and validated using two sinusoidal temperature profiles (5 - 15ºC and 10 - 40ºC). The accuracy and bias factors for both profiles were within the acceptable range (0.75 – 1.25), and more than 92% of the predicted errors between predicted and growth observations were within the acceptable prediction zone (-0.5 to 1.0 log10 CFU/g). The newly developed dynamic predictive model can be used by the food industry as well as regulatory agencies to predict *B. cereus* spore germination and outgrowth in roast hamduring processing, extended storage, and distribution, even under temperature conditions and consequently help to avoid foodborne diseases caused by *B. cereus*.

Introduction

Bacillus cereus is a spore-forming, motile, facultative anaerobe and gram-positive bacterium that is commonly found in the environment as well as variety of food (Granum, 1994). This pathogen has been incriminated for causing two types of food poisoning, diarrheal and emetic

(Drobniewski, 1993). Illness can occur following ingestion of food stored at improper temperatures for several hours before serving or inadequate rapid cooling of food after high-temperature cooking (Adams and Moss, 2000; Mossel et al., 1995). Diarrhea and vomiting are the common symptoms due to the release of heat-labile diarrheal enterotoxin, and heat-stable emetic enterotoxin respectively (Stenfors Arnesen et al., 2008). The United States Centers for Disease Control and Prevention (CDC) estimated that 20 hospitalizations and 63,400 cases of foodborne illness occur annually in the United States due to *B. cereus* (Scallan et al., 2011).

Poultry and meat products are generally involved in most of the *B. cereus* food-borne illness outbreaks (Bennett et al., 2013). Self et al. (2017), analyzed the epidemiology of outbreaks associated with pork in the United States that have been reported to CDC from 1998 to 2015 (Self et al., 2017). They reported 288 outbreaks in total due to all the major foodborne pathogens that resulted in 6372 illnesses and 443

hospitalizations. Out of all of them, *B. cereus* was responsible for 15 of the outbreaks that resulted in 12 hospitalizations and the highest median number of illnesses, 195 in different food types, such as ham, entrée, barbecue, processed and by-products of pork. However, most of the *B. cereus* food-borne illness cases are not reported or diagnosed, as the symptoms can be mild or short-lasting symptoms.

The spore-forming ability of *B. cereus* makes it highly resistant to environmental stresses such as heat or radiation, low pH values or chemical conservation (Nicholson et al., 2000; Setlow, 2006). Further, the ability of the bacterium to form biofilms on the surfaces in the food processing plants complicates cleaning and disinfection measures, especially in the piping systems of food processing plants (Karunakaran and Biggs, 2011; Peng et al., 2002; Ryu and Beuchat, 2005).

Predictive modeling programs such as ComBase and the Pathogen Modeling Program (PMP) by

the United States Department of Agriculture, Agricultural Research Service (USDA-ARS) are extensively used by the regulatory agencies and food industries to predict the growth of *B. cereus*. However, the predictive models offered by these user-friendly modeling tools were developed using liquid microbiological media and have not been validated in food matrices. Moreover, ComBase and PMP growth model provide predictions for the growth of *B. cereus* up to 34ºC and 42ºC respectively. But this bacterium can grow from 4 to 55ºC. So, they are not fail-safe for lag phase predictions and growth over the whole biokinetic range. Currently, there are three dynamic predictive models available in literature for the growth of *B. cereus* spores in beans, rice, and pasta (Juneja et al., 2017a; Juneja et al., 2019a). The goal of this study was to develop and validate a dynamic model, estimating the growth of *B. cereus* spores in ham. The developed dynamic predictive model for ham can be useful for regulatory agencies such as USDA-FSIS and meat processing plants to address the public health concern about the outgrowth and germination of *B. cereus* and its production of emetic and diarrheal toxins in roast ham under conditions of temperature abuse due to improper cooling rates or improper storage conditions.

METHODS AND MATERIALS

Bacillus cereus strains and its spore cocktail generation

Four strains of *Bacillus cereus* were obtained from the Center for Food Safety, UGA. All the four strains are diarrheal toxin producing strains which include *B. cereus* F4810/72, isolated from cooked rice, *B. cereus* F4512A/87 isolated from pasteurized milk, *B. cereus* 038-2 isolated from infant formula, and *B. cereus* F3812/84 isolated from pasteurized milk. All the strain stock culture were maintained at 80ºC till use in cryobeads (Pro-Lab Diagnostics™ Microbank™, PL.170C/G, Thermo Fisher Scientific, USA).

B. cereus spores were prepared following the protocol demonstrated by Juneja et al.,

(2017). Active vegetative cells were obtained by inoculating a bead of each strain into sterile brain heart infusion broth tubes (10mL, BHI) broth and incubated overnight at 37ºC. Subsequently, 100 µL of each active cell suspension was surface plated on thirty nutrient agar (Difco, BD, Sparks, MD and Fisher Scientific) containing manganese sulfate (0.05g/L $MnSO_4$; Waltham, Massachusetts, USA) (NAMS) plates. The plates were incubated for 10 d at 37ºC. Sterile distilled water (5mL) was added to each NAMS agar plate, and the spores were carefully scraped with sterile plastic spreaders and were collected into Falcon® tubes (50mL) and centrifuged (Centrifuge 5804, Eppendorf, Hamburg, Germany) at 10,000 g for 15 minutes at 4ºC. The pellet was resuspended in sterile distilled water (10mL) after discarding the supernatant. The suspension was centrifuged again as described earlier. This process was repeated twice before the final pellet was resuspended in 10 mL of sterile distilled water. Each spore suspension was then stored at -20ºC until further use. The population of *B. cereus* spores for each strain was determined by subjecting the spores to heat treatment for 10 min at 80ºC. The spores were then serially diluted using sterile peptone water (0.1% w/v; PW) and plated on Tryptic Soya Agar (TSA; Difco, Detroit, Michigan, USA) with yeast extract (6 g/L; BBL, Farnklin Lakes, New Jersy, USA) agar plates. The plates were incubated for 24 h at 37ºC, and the *B. cereus* colonies were enumerated. Finally, a four-strain *B. cereus* spore cocktail was prepared by combining equal amounts of spores from each strain's spore crop (Juneja et al., 2017a).

Meat preparation and spore inoculation

The vacuum packaged ham meat was transported under refrigeration and sourced from a commercial pork processor. The ham was cut into 2.5 cm cubes and coarsely ground using a 19 mm grinder plate in a meat grinder (PC–98/32, Mainca USA, ST. Louis, Missouri, USA). A marinade consisting of water, salt (1%; Custom blended seasonings, A. C. Legg, Inc., Calera,

Alabama, USA) and phosphate (0.3%; Sodium polyphosphate, Brifisol® 512, Ettlinger Corporation, Kansas, USA) based on the final weight of the product, were added to the ground meat, which was then mixed for 15 min using a meat mixer (RM -20INT, Mainca USA, ST. Louis, Missouri, USA). The marinated meat was finely ground using a 6.35 mm plate (PC–98/32, Mainca USA, ST. Louis, Missouri, USA). The ground meat was vacuum sealed (Multivac Sepp Haggenmuller GmbH and Co. KG, Wolferrschwenden, Germany) and stored at -20ºC until further use.

The ground ham meat was thawed overnight and portioned into vacuum bags (5g) (Ultravac 21/2" x 10" Chamber Vacuum Packaging Pouches 3 mil, UltraSource LLC, Kansas City, Missouri, USA). Each bag was then inoculated with 50µL of a *B. cereus* spore cocktail to achieve a concentration of approximately 2.5 log CFU/g of spores. Negative controls were also prepared by inoculating 50µL of 0.1% sterile peptone water (PW) without microbial spores. Air was removed from all the pouches by manual pressing and heat-sealed (Impulse heat sealer, AIE,
Long Beach, California, USA) to maintain the natural texture of the meat. The sealed meat packs were thoroughly massaged for 2 minutes to ensure uniform mixing of the spores. Finally, the heat-sealed meat packs were subjected to a heat treated for 10 min at 80**º**C using a circulating water bath (Model MX20H135-A11B Heated Circulating Water Bath, PolyScience, Illinois, USA) and transferred to water baths set at specific temperatures (Juneja et al., 2018).

pH and aw

The pH and water activity (a_W) of the prepared ground ham meat were assessed using a pH meter (pHTestr® 5F, Oakton, Long Beach, New Jersey, USA) and water activity meter (Dew Point Water Activity Meter 4TE, AquaLab, Pullman, Washington, USA), respectively following manufacturer's instructions.

B. cereus growth under isothermal and sinusoidal temperature profiles

The growth date for *B. cereus* was collected at various isothermal temperatures by submerging the heat-treated meat bags in a circulating water bath (MX20H135-A11B Circulating Water Bath, MX Controller, PolyScience, Illinois, USA) set at temperatures ranging from 10ºC to 55ºC including the entire bio-kinetic growth range of *B. cereus*. Each storage temperature underwent three independent replications. At predetermined sampling points, one sample per replication (~15 samples/replication) was collected and the population of *B. cereus* was enumerated. The storage duration ranged from 15 d at 50ºC to 32 h at 30ºC to 40 d at 10ºC.

Two dynamic sinusoidal profiles were used, and *B. cereus* growth was determined as described to validate the developed model. The heat-treated meat bags were transferred to a programmable chilling water bath (AP15R-40-A11B, Polyscience, Illinois, USA) with continuous water circulation. The chiller was programmed to follow a non-isothermal temperature profile ranging from 5 to 15ºC (6 h cycle) or 10 to 40ºC (5 h cycle) for 27 d and 36 h respectively (Singh et al., 2011). The meat samples were removed at predetermined sampling points and *B. cereus* density was enumerated as described below. For each temperature profile, three independent replications were performed.

B. cereus enumeration

The meat samples were collected at each designated sampling time from the water bath and each meat block was placed aseptically in a filter bag (B01385, Whirl-Pak Filter bag, Nasco, Fort Atkinson, Wisconsin, USA). To each bag, PW (20mL) was added. The meat was stomached (Basic paddle maxicator, Neutec Group Inc., New York, USA) for a duration of 2 min and serially diluted the suspension in PW and plated on Petrifilms™ (3M, St. Paul, Minnesota, USA). Negative controls (non-inoculated) were plated to check the absence of naturally

occurring *B. cereus* (Juneja et al., 2018, 2020).

PREDICTIVE MODELLING

Primary growth model

The Baranyi model is a widely used mathematical tool to analyze the growth of microorganisms under various environmental variables such as temperature (Juneja et al., 2018). Baranyi and Roberts developed the Baranyi model to depict the growth of *B. cereus* over time for different temperatures. This model uses the following two equations

$$\frac{dy}{dt} = \frac{1}{1+e^{-Q\mu max}}\left(1 - e^{(y-y_{max})}\right) \quad \text{.....(1.1)}$$

$$\frac{dQ}{dt} = \mu_{max} \quad \text{.....(1.2)}$$

The Baranyi model, which has four parameters, was used to characterize bacterial growth in an isothermal environment. The two equations were solved to describe this growth, with specific starting conditions of $y = y_0$ at $t = 0$ and $Q = \log_e(q_o)$, where q stands for the early physiological state of the microbial cell populations.

$$y^{(t)} = y_0 + \mu_{max} F(t) - \log_e\left(1 + \frac{e^{\mu_{max} F(t)} - 1}{e^{(y_{max} - y_0)}}\right) \quad \text{.....(2)}$$

Where the function

$$F(t) = t + \frac{1}{v}\log_e\left(e^{-vt} + e^{-h_0} - e^{(-vt-h_0)}\right) \quad \text{.....(3)}$$

The variable y(t) represents the natural logarithm of the concentration, indicating the *B. cereus* population in CFU/g at a given time. The parameters y_0 and y_{max} correspond to the initial and maximum population sizes of *B. cereus* in units of ln CFU/g, respectively. The maximum specific growth rate (μ_{max}, ln CFU/h) and is considered equivalent to the limiting rate of increase, *v*. The equation $h_0 = (\mu_{max})(\lambda)$, where λ represents the length of the lag period (h) and is

calculated using the following formula.

$$\lambda = \frac{h_0}{\mu_{max}} \quad \text{.....(4)}$$

Singh et al. (2011) conducted an analysis of the model by fitting the growth data in ln CFU/mL units, which were ultimately converted to $\log_{10}$ CFU/mL units. The growth data was plotted against time, and parameters such as h_0, y_0, y_{max}, and μ_{max} were estimated for each temperature point. The lower and upper asymptotes, y_0 and y_{max}, were considered as the initial and final population density respectively. The specific maximum growth rate (μ_{max}) was obtained by calculating the slope of the exponential stage of bacterial count over time. The initial value of h_0 was obtained by multiplying the lag time (λ) with the maximum specific growth rate (μ_{max}). The lag time represents the time interval between the start of the growth and the initiation of exponential growth (Singh et al., 2011).

To demonstrate the sigmoidal growth of *B. cereus*, growth data obtained at different temperature points were fitted using the curve fitting tool in MATLAB software (Version R2018a, MathWorks, Natick, MA) (Huang, 2013; Mahdinia et al., 2020; M. Thomas et al., 2019). Therefore, in the initial estimation of the Baranyi model, four parameters (h_0, y_0, y_{max}, and μ_{max}) were determined (Baranyi and Roberts, 1994; Buchanan et al., 1997; Juneja et al., 2017a). The average value of h_0 was calculated from the models and treated as a constant because the pre-inoculation history of all bacterial cells is consistent. While the other three parameters (y_0, y_{max}, and μ_{max}) were re-estimated by re-fitting them into the Baranyi model (Huang, 2008, 2013; Juneja et al., 2008a, 2017b).

Secondary Model

Secondary model was used to fit the primary model parameters (y_0, y_{max}, and μ_{max}) as a function of temperature to establish the correlation between the estimates of μ_{max} and the corresponding

temperatures (Juneja et al., 2009, 2021). The secondary model accounts for a variety of biotic and abiotic factors that can influence microbial growth kinetics, such as temperature, water activity, pH, and other environmental conditions (Baranyi and Roberts, 1994; Gonçalves et al., 2017; Xanthiakos et al., 2006). In this study, temperature was the only variable factor, remaining elements such as pH, a_w were constant as the same product was used. The Ratkowsky model was particularly used as a secondary model to fit maximum specific growth rates (μ_{max}) to assess the effect of temperature on μ_{max} (Geeraerd et al., 2004; Ratkowsky et al., 1982, 1983; Ross, 1996; Zwietering et al., 1991, 1996).

$$\mu_{max} = a(T - T_{min})^2 \{1-\exp[b(T - T_{max})]\} \quad (5)$$

The equation considers the temperature variable T (in degrees Celsius), as well as the minimum (T_{min}) and maximum (T_{max}) growth limits in degrees Celsius. Regression analysis is used to calculate the coefficients a and b. Furthermore, at each temperature, the lag time was modeled using a hyperbolic function (Zwietering et al., 1991, 1996).

$$\lambda = \frac{p}{e^{T-q}} \quad (6)$$

The parameter p represents the rate at which the lag phase length decreases with increasing temperature, whereas the parameter q represents the temperature at which the lag phase length becomes infinite. T represents the temperature in degrees Celsius. To construct secondary models incorporating these parameters, the curve fitting toolbox in MATLAB software (Version R2018a, MathWorks, Natick, MA) was used, with the trust-region algorithm for non-linear least squares (Juneja et al., 2019a; Juneja et al., 2019a; Mahdinia et al., 2020).

Primary and secondary model evaluation - Goodness of fit statistics

The performance of both primary and secondary models was evaluated using goodness of fit

statistics. Accuracy and bias factors (Ross, 1996), coefficient of determination (R^2) (Di Bucchianico, 2008), and root mean square error (RMSE) (Hodson, 2022) were used to evaluate performance. These statistical measures were used to compare the primary and secondary model performances as well as the developed dynamic model (Juneja et al., 2021; Juneja et al., 2019a). To assess the model's performance further, the coefficient of determination, R^2 was calculated using the following equation.

$$R^2 = \frac{SSR}{SSTO} \quad \text{.....(7)}$$

where

$$SSR = \sum_{i=1}^{n} \left(\hat{y}_i - \bar{y}\right)^2 \quad \text{.....(7.1)}$$

and

$$SSTO = \sum_{i=1}^{n} \left(y_i - \bar{y}\right)^2 \quad \text{.....(7.2)}$$

SSR and SSTO are the regression and total sum of squares, respectively. The goodness of fit statistics was computed using the MATLAB software. The following equation was used to calculate the RMSE value (Chai and Draxler, 2014).

$$\sqrt{\frac{1}{n}\sum_{i=1}^{n} e_i^2} \quad \text{.....(8)}$$

The formula incorporates the following variables: n denotes the sample size, $\hat{y}$ represents the predicted regression, $\bar{y}$ signifies the mean response variable, and y represents the response variable, specifically the population density of *B. cereus*.

$$\text{Accuracy factor} = 10^{\frac{\sum\left|\log\left(\frac{GT_{predicted}}{GT_{observed}}\right)\right|}{n}} \quad \text{.....(9)}$$

$$\text{Bias factor} = 10^{\frac{\sum \log\left(\frac{GT_{predicted}}{GT_{observed}}\right)}{n}} \quad \text{.....(10)}$$

The variable "$GT_{predicted}$" corresponds to the predicted generation time (measured in h),

"$GT_{observed}$" corresponds to the observed generation time (measured in h), and "n" represents the sample size.

Tertiary Model

Tertiary models were created by combining primary and secondary models using the MATLAB software (Butcher, 1996; Velugoti et al., 2011). The combination of these models enables a thorough understanding of the growth dynamics. Baranyi and Roberts (1994) pioneered the use of first-order differential equations to describe differential growth (Baranyi and Roberts, 1994).

$$\frac{dy}{dt} = \frac{1}{1+e^{-Q(t)}} \mu_{max}[T(t)]\left(1-e^{y(t)-y_{max}}\right) \quad \text{.....(11)}$$

$$\frac{dQ}{dt} = \mu_{max}[T(t)] \quad \text{.....(12)}$$

where

$Q(t)=\ln q(t)$

The starting conditions of the model are characterized by y_0 and Q_0, whereby y_0 denotes the initial population density of *B. cereus*, and Q_0 represents the initial physiological state of the cells. The value of Q_0 is obtained by taking the natural logarithm of q_0. The differential equations of the model were solved using the MATLAB software and the fourth order Runge-Kutta method, as proposed by Butcher in 1996. By incorporating dynamic time-temperature profiles, the model utilized its calculations to predict the *B. cereus* density (Gumudavelli et al., 2007; Velugoti et al., 2011).

Model Validation

To developed tertiary model were validated by conducting experiments using dynamic time-temperature profiles: 10 to 40ºC for 37 h and 5 to 15ºC for 27 d ((Singh et al., 2011). Inoculated meat was prepared, and *B. cereus* density was determined as described previously at pre-

determined sampling points to include growth of *B. cereus* till it reach stationary phase.

The accuracy of the Baranyi model's predictions for each dynamic profile was evaluated using Acceptable Prediction Zone (APZ) analysis, a method previously employed in similar studies (T. E. Oscar, 2005; T. P. Oscar, 2005; Wang et al., 2017). Prediction errors (PE), calculated by comparing the predicted values with the observed values in $\log_{10}$ CFU/g, served as indicators of accuracy. Positive PE values indicated fail-dangerous predictions, negative PE values suggested fail-safe predictions, and a PE value of 0 signified a perfect prediction (T. E. Oscar, 2005; T. P. Oscar, 2005). The acceptable prediction zone (APZ) limits were defined as -1.0 to 0.5 $\log_{10}$ CFU/g, within which the predictions were considered acceptable (Juneja et al., 2017c; M. Thomas et al., 2019; L. V Thomas and Isak, 2005).

RESULTS AND DISCUSSION

pH and water activity

The pH values of the prepared ham and roast ham roast (before and after heat treatment) were 5.98± 0.005 and 6.03 ± 0.02, respectively. These pH values of the ham were in close agreement with the previous literature (Guerrero et al., 1999).

The water activity values before and after heat treatment (prepared ham and roast ham roast) were 0.9876 ± 0.004 and 0.9860 ± 0.002, respectively. The minimum water activity required for the growth of *B. cereus* is 0.9510 and the water activity of the ham roast was suitable for *B. cereus* growth. No significant difference observed in pH and a_w values between before and after heat treatments.

Primary model

B. cereus growth data was collected from each isothermal temperature and was fitted to primary Baranyi model (Fig. 5.1). The h_0 values ranged from 3.88×10^{-13} to 5.35, with a mean of 1.34.

Consequently, the h_0 value was standardized at 1.34. The mean h_0 value varied among different pathogens in various food matrices. For instance, in roast ham, *Clostridium perfringens* exhibited an h_0 value of 6.7, while *Salmonella* in chicken had an h_0 value of 1.7. *B. cereus* had an h_0 value of 4.1 in cooked rice, 3.46 in cooked pasta, and 4.80 in cooked beans (Amezquita et al., 2005; Juneja et al., 2007, 2017a; Juneja et al., 2019b, 2019c; Juneja et al., 2019b). *Brochothrix thermosphacta* showed an average h_0 value of 3.2 in broth (Baranyi et al., 1995). The variations in h_0 values reported in previous studies are likely due to differences in the strains of microorganism(s) tested and the growth media (food product [s]) used.

The Baranyi model demonstrated good performance with a high R^2 value of 0.9973 and a low RMSE value of 0.2392 log CFU/g across all growth temperatures. These results indicate the model's ability to predict *B. cereus* growth, as supported by previous studies (Juneja et al., 2008b, 2011; Juneja et al., 2019a). Table - 5.1 provides an overview of the goodness of fit for each primary growth model at each consistent growth temperature. Fig. 5.1 illustrates the observed and predicted growth of *B. cereus* at various consistent temperatures when the *B. cereus* growth data was fitted to the primary growth model.

Insert Figure-1

Secondary model

The research incorporated primary model's kinetic parameters, such as lag phase duration and growth rate, into secondary models like the Ratkowsky model. This secondary model proved to be a good fit for describing the observed maximum specific growth rate (μ_{max}) of *B. cereus* in roast

ham as a function of temperature (Fig. 2). The Ratkowsky model estimated theoretical T_{min} (12.38ºC) and T_{max} (50.11ºC), which is like the observed T_{min} (11.37ºC) and T_{max} (50.44ºC). The

predicted T_{min} and T_{max} values for roast ham in this study were like those predicted for cooked pasta (8.99 and 49.92, respectively) and cooked rice (8.69 and 49.68, respectively), as indicated by Juneja, Golden, Mishra, Harrison, and Mohr in 2019 and Juneja, Golden, Mishra, Harrison, Mohr, et al. in 2019. The estimated values aligned with the observed T_{min} (10ºC) and T_{max} (50ºC) values which were almost in accordance with *B. cereus* in cooked rice (13 and 46ºC), cooked pasta (13 and 46ºC), cooked beans (13 and 46ºC) and with *C. perfringens* in cooked uncured beef (13 and 51ºC) (Juneja et al., 2008b, 2017a; 2019a).

The modified Ratkowsky model successfully estimated the maximum specific growth rate of *B. cereus* in roast ham, as evidenced by the goodness of fit statistics. The model closely matched the observed μ_{max} values for each primary model, with a high R^2 value of 0.9435 and a low RMSE of 0.0537 $\log_{10}$ CFU/g. According to the secondary Ratkowsky model, the growth rate increased with temperature up to 35ºC and then decreased from 40ºC to 50ºC (refer Fig. 5.2). The maximum growth rate observed at 35ºC was 0.6153 $\log_{10}$ CFU/h, which was consistent with the maximum growth rate of *B. cereus* in cooked beans (0.6 log CFU/h). The minimum growth rate in ham observed at 50ºC was 0.0044 $\log_{10}$ CFU/h, while in cooked beans, it was 0.17 log CFU/g at 13ºC (Juneja et al., 2017a).

Insert figure – 5.2 and table – 5.1.

Tertiary/ Dynamic models and Validation:

Tertiary models are computational tools widely used in the food industry and research that combine primary and secondary models. These models employ algorithms to calculate changing conditions and provide predictions. Validation of the growth models in roast ham was conducted using dynamic temperature profiles ranging from 5ºC to 15ºC and 10ºC to 40ºC (Fig. 5.3). The inputs for this model were derived from the fitted primary and secondary models to predict the

growth. The parameters from the primary growth model (Table – 5.2), including y_0 (2.58), y_{max} (7.82), and h_0 (1.34), along with parameters from the secondary models such as T_{min} (12.38ºC), T_{max} (50.11ºC), a (0.9156), and b (0.0001), were utilized in the tertiary models through the 4th order Runge-Kutta method.

Dynamic models were employed to validate the developed models, as depicted in Figure 3, which represents the dynamic models for two sinusoidal temperature profiles (5ºC to 15ºC and 10ºC to 40ºC) alongside the observed growth from the validation experiments. In both profiles, the experimentally observed maximum population density matched the predicted maximum population density, similar to those for *Salmonella* spp. in liquid whole egg (Singh et al., 2011), but unlike the observations for other bacteria such as *Listeria monocytogenes* in queso fresco (M. Thomas et al., 2019). The evaluation of the validated model was performed using the acceptable prediction zone (APZ) analysis method, and the prediction errors indicated that the tertiary models were deemed acceptable.

The current study revealed that all observed points except one observation from the high-temperature profile and 44 out of 50 points from the low-temperature profile fell within the APZ limits, where the prediction error ranged between 0.5 and -1.0 $\log_{10}$ CFU/g, as illustrated in Fig. 4A. An acceptable model typically exhibits a prediction error percentage within 70%. In this study, more than 92% of the predicted errors between predicted and growth observations fell within the acceptable prediction zone (-0.5 to 1.0 $\log_{10}$ CFU/g). The asymmetric margins in the APZ analysis are since prediction errors (Log_{10} CFU/g) can be tolerated if they are in the negative direction (fail-safe), as shown in Fig. 5.4. Additionally, an excessively fail-safe model would result in the unnecessary disposal of food that is safe for consumption, while an excessively fail-dangerous model (PE beyond 0.5 $\log_{10}$ CFU/g) would lead to the consumption of

unsafe foods, potentially causing outbreaks or illnesses. Low sinusoidal profile showed that most of the predictions matched the observations, except for certain time points ranging from 350 to 500 hours, where the observed values were slightly towards the positive side (fail-dangerous), opposite to the observations reported by Singh et al., (2011) (Fig. 5.4). In contrast, the observed values and predicted values were perfectly matched with each other in high sinusoidal temperature profile, which indicates there is no point of fail-safe and fail-dangerous Fig 5.4.

Insert Figure 5.3 and figure 5.4

Conclusion

The dynamic models developed using the Baranyi, Ratkowsky, and tertiary models demonstrated excellent performance, with all goodness-of-fit parameters falling within acceptable ranges. This indicates that regulatory agencies and food processors can utilize these models to predict the growth of *B. cereus* in ready-to-eat (RTE) roast ham across various storage and temperature conditions, thereby ensuring food safety. Moreover, these models assist in determining appropriate cooling rates for cooked meat to prevent the germination and outgrowth of *B. cereus* spores. Predictive models are gaining popularity and are being integrated into extensive databases to support industries and organizations in guaranteeing food safety and preservation. The next generation of models is expected to enhance safety assurance, contribute to shelf-life control, and facilitate hazard analysis procedures (HACCP), among other areas. Ultimately, these endeavors will play a crucial role in preventing foodborne illnesses caused by *B. cereus.*

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sReferences

Adams, M. R., & Moss, M. O. (2000). *Bacillus cereus* and other *Bacillus* species. *Food Microbiology. MPG Books Ltd., Bodmin, UK*, 187–192.

Amezquita, A., Weller, C. L., Wang, L., Thippareddi, H., & Burson, D. E. (2005). Development of an integrated model for heat transfer and dynamic growth of *Clostridium perfringens* during the cooling of cooked boneless ham. *International Journal of Food Microbiology*, *101*(2), 123–144.

Baranyi, J., & Roberts, T. A. (1994). A dynamic approach to predicting bacterial growth in food. *International Journal of Food Microbiology*, *23*(3–4), 277–294.

Baranyi, J., Robinson, T. P., Kaloti, A., & Mackey, B. M. (1995). Predicting growth of *Brochothrix thermosphacta* at changing temperature. *International Journal of Food Microbiology*, *27*(1), 61–75.

Bennett, S. D., Walsh, K. A., & Gould, L. H. (2013). Foodborne disease outbreaks caused by *Bacillus cereus*, *Clostridium perfringens*, and *Staphylococcus aureus*—United States, 1998–2008. *Clinical Infectious Diseases*, *57*(3), 425–433.

Buchanan, R. L., Whiting, R. C., & Damert, W. C. (1997). When is simple good enough: a comparison of the Gompertz, Baranyi, and three-phase linear models for fitting bacterial growth curves. *Food Microbiology*, *14*(4), 313–326.

Butcher, J. C. (1996). A history of Runge-Kutta methods. *Applied Numerical Mathematics*, *20*(3), 247–260.

Chai, T., & Draxler, R. R. (2014). Root mean square error (RMSE) or mean absolute error (MAE)? –Arguments against avoiding RMSE in the literature. *Geoscientific Model*

Development, *7*(3), 1247–1250.

Drobniewski, F. A. (1993). *Bacillus cereus* and related species. *Clinical Microbiology Reviews*, *6*(4), 324–338.

Geeraerd, A. H., Valdramidis, V. P., Devlieghere, F., Bernaert, H., Debevere, J., & Van Impe, J. F. (2004). Development of a novel approach for secondary modelling in predictive microbiology: incorporation of microbiological knowledge in black box polynomial modelling. *International Journal of Food Microbiology*, *91*(3), 229–244.

Granum, P. E. (1994). *Bacillus cereus* and its toxins. *Journal of Applied Microbiology*, *76*(S23), 61S-66S.

Guerrero, L., Gou, P., & Arnau, J. (1999). The influence of meat pH on mechanical and sensory textural properties of dry-cured ham. *Meat Science*, *52*(3), 267–273.

Gumudavelli, V., Subbiah, J., Thippareddi, H., Velugoti, P. R., & Froning, G. (2007). Dynamic predictive model for growth of *Salmonella enteritidis* in egg yolk. *Journal of Food Science*, *72*(7). https://doi.org/10.1111/j.1750-3841.2007.00444.x

Huang, L. (2008). Growth kinetics of *Listeria monocytogenes* in broth and beef frankfurters—determination of lag phase duration and exponential growth rate under isothermal conditions. *Journal of Food Science*, *73*(5), E235–E242.

Huang, L. (2013). Optimization of a new mathematical model for bacterial growth. *Food Control*, *32*(1), 283–288.

Juneja, V. K., Golden, C. E., Mishra, A., Harrison, M. A., & Mohr, T. B. (2019a). Predictive model for growth of *Bacillus cereus* at temperatures applicable to cooling of cooked pasta. *Journal of Food Science*, *84*(3), 590–598.

Juneja, V. K., Golden, C. E., Mishra, A., Harrison, M. A., Mohr, T., & Silverman, M. (2019a).

Predictive model for growth of *Bacillus cereus* during cooling of cooked rice. *International Journal of Food Microbiology, 290*, 49–58.

Juneja, V. K., Marks, H., Huang, L., & Thippareddi, H. (2011). Predictive model for growth of *Clostridium perfringens* during cooling of cooked uncured meat and poultry. *Food Microbiology*, *28*(4), 791–795. https://doi.org/10.1016/j.fm.2010.05.013

Juneja, V. K., Marks, H., & Thippareddi, H. (2008a). Predictive model for growth of *Clostridium perfringens* during cooling of cooked uncured beef. *Food Microbiology*, *25*(1), 42–55. https://doi.org/10.1016/j.fm.2007.08.004

Juneja, V. K., Melendres, M. V., Huang, L., Gumudavelli, V., Subbiah, J., & Thippareddi, H. (2007). Modeling the effect of temperature on growth of *Salmonella* in chicken. *Food Microbiology*, *24*(4), 328–335.

Juneja, V. K., Mishra, A., & Pradhan, A. K. (2017a). Dynamic predictive model for growth of *Bacillus cereus* from spores in cooked beans. *Journal of Food Protection*, *81*(2), 308–315.

Juneja, V. K., Osoria, M., Hwang, C.-A., Mishra, A., & Taylor, T. M. (2020). Thermal inactivation of *Bacillus cereus* spores during cooking of rice to ensure later safety of boudin. *LWT*, *122*, 108955.

Juneja, V. K., Purohit, A. S., Golden, M., Osoria, M., Glass, K. A., Mishra, A., Thippareddi, H., Devkumar, G., Mohr, T. B., Minocha, U., Silverman, M., & Schaffner, D. W. (2021). A predictive growth model for *Clostridium botulinum* during cooling of cooked uncured ground beef. *Food Microbiology, 93*. https://doi.org/10.1016/j.fm.2020.103618

Mahdinia, E., Liu, S., Demirci, A., & Puri, V. M. (2020). Microbial growth models. *Food Safety Engineering*, 357–398.

Mossel, D. A. A., Corry, J. E. L., Struijk, C. B., & Baird, R. M. (1995). *Essentials of the*

microbiology of foods: a textbook for advanced studies. John Wiley & Sons.

Nicholson, W. L., Munakata, N., Horneck, G., Melosh, H. J., & Setlow, P. (2000). Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. *Microbiology and Molecular Biology Reviews*, *64*(3), 548–572.

Oscar, T. E. (2005). Validation of lag time and growth rate models for *Salmonella Typhimurium*: acceptable prediction zone method. *Journal of Food Science*, *70*(2), M129–M137.

Oscar, T. P. (2005). Development and validation of primary, secondary, and tertiary models for growth of *Salmonella Typhimurium* on sterile chicken. *Journal of Food Protection*, *68*(12), 2606–2613.

Ratkowsky, D. A., Lowry, R. K., McMeekin, T. A., Stokes, A. N., & Chandler, R. (1983). Model for bacterial culture growth rate throughout the entire biokinetic temperature range. *Journal of Bacteriology*, *154*(3), 1222–1226.

Ratkowsky, D. A., Olley, J., McMeekin, T. A., & Ball, A. (1982). Relationship between temperature and growth rate of bacterial cultures. *Journal of Bacteriology*, *149*(1), 1–5.

Ross, T. (1996). Indices for performance evaluation of predictive models in food microbiology. *Journal of Applied Bacteriology*, *81*(5), 501–508.

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V, Widdowson, M.-A., Roy, S. L., Jones, J. L., & Griffin, P. M. (2011). Foodborne illness acquired in the United States—major pathogens. *Emerging Infectious Diseases*, *17*(1), 7–15.

Self, J. L., Luna-Gierke, R. E., Fothergill, A., Holt, K. G., & Vieira, A. R. (2017). Outbreaks attributed to pork in the United States, 1998–2015. *Epidemiology & Infection*, *145*(14), 2980–2990.

Setlow, P. (2006). Spores of *Bacillus subtilis*: their resistance to and killing by radiation, heat

and chemicals. *Journal of Applied Microbiology*, *101*(3), 514–525.

Singh, A., Korasapati, N. R., Juneja, V. K., Subbiah, J., Froning, G., & Thippareddi, H. (2011). Dynamic predictive model for the growth of *Salmonella* spp. in liquid whole egg. *Journal of Food Science*, *76*(3), M225–M232.

Thomas, M., Tiwari, R., & Mishra, A. (2019). Predictive model of *Listeria monocytogenes* growth in queso fresco. *Journal of Food Protection*, *82*(12), 2041–2079. https://doi.org/10.4315/0362-028X.JFP-19-185

Thomas, L. V, & Isak, T. (2005). Nisin synergy with natural antioxidant extracts of the herb rosemary. *I International Symposium on Natural Preservatives in Food Systems 709*, 109–114.

Velugoti, P. R., Bohra, L. K., Juneja, V. K., Huang, L., Wesseling, A. L., Subbiah, J., & Thippareddi, H. (2011). Dynamic model for predicting growth of *Salmonella* spp. in ground sterile pork. *Food Microbiology*, *28*(4), 796–803.

Wang, J., Koseki, S., Chung, M.-J., & Oh, D.-H. (2017). A novel approach to predict the growth of *Staphylococcus aureus* on rice cake. *Frontiers in Microbiology*, *8*, 1140.

Zwietering, M. H., De Koos, J. T., Hasenack, B. E., De Witt, J. C., & Van't Riet, K. (1991). Modeling of bacterial growth as a function of temperature. *Applied and Environmental Microbiology*, *57*(4), 1094–1101.

Zwietering, M. H., De Wit, J. C., & Notermans, S. (1996). Application of predictive microbiology to estimate the number of *Bacillus cereus* in pasteurised milk at the point of consumption. *International Journal of Food Microbiology*, *30*(1–2), 55–70.

Table 5.1: Specific growth rate (μ_{max}), lag-time duration (λ), maximum population density (y_{max}) and goodness of fit parameters such as R^2 and RMSE values at each isothermal temperature obtained after fitting *B. cereus* growth data in ham to the primary (Baranyi) model.

Temperature (ºC)	μ_{max}	y_o	y_{max}	R2	RMSE
15	0.07	2.83	7.51	0.9828	0.2654
20	0.10	2.69	8.07	0.9924	0.1968
25	0.43	2.41	8.13	0.9770	0.3683
30	0.56	2.44	7.79	0.9859	0.2722
35	0.65	2.47	7.73	0.9834	0.2673
40	0.65	2.68	7.65	0.9923	0.1772
45	0.53	2.49	7.65	0.9971	0.1167
50	0.04	2.60	8.00	0.9871	0.2494

Table 5.2: Primary (Baranyi) and secondary (modified Ratkowsky) model parameters used to develop tertiary models using 4th order Runge-Kutta method

Models and Parameters	**Values**
Primary Model	
y_0 (log CFU/g)	2.58
y_{max} (log CFU/g)	7.82
h_0	1.34
Secondary Model	

T_{min} (°C)	12.38
T_{max} (°C)	50.11
a	0.9156
b	0.0000

Figure 5.1: Growth of *B. cereus* (Log CFU/g) in roast ham at various isothermal temperatures fitted to the primary (Baranyi) model.

(Predicted growth: [ ]; Observed growth:

Replication-1=[□]; Replication-2=[□]; and Replication-3=[•]

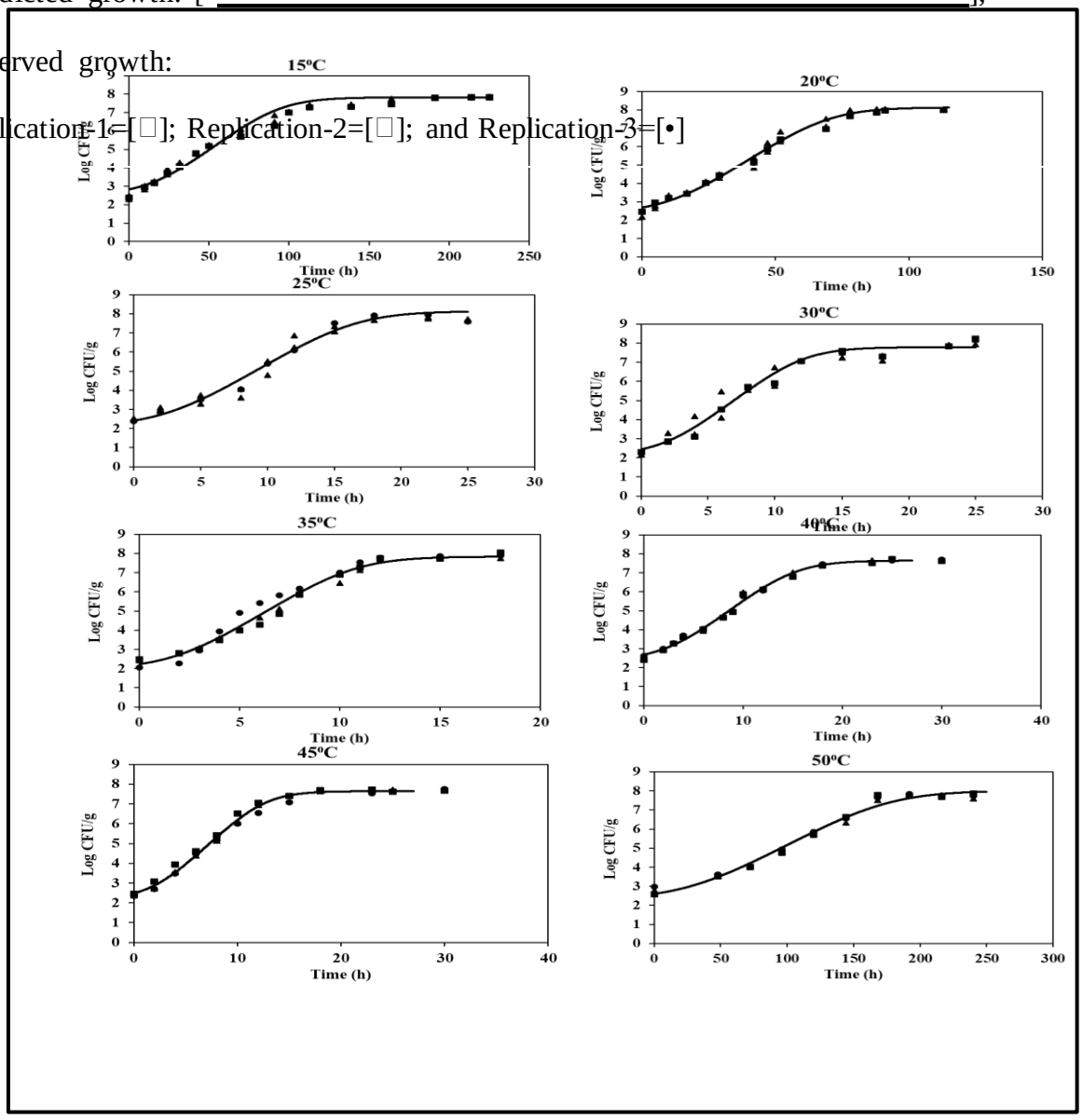


Figure 5.2: Secondary (modified Ratkowsky) model describing the growth rate (log CFU/h) of B. cereus in ham as a function of temperature (ºC), compared to growth rate of B. cereus rates in RTE turkey breast, ham, cooked beans, pasta, and rice.

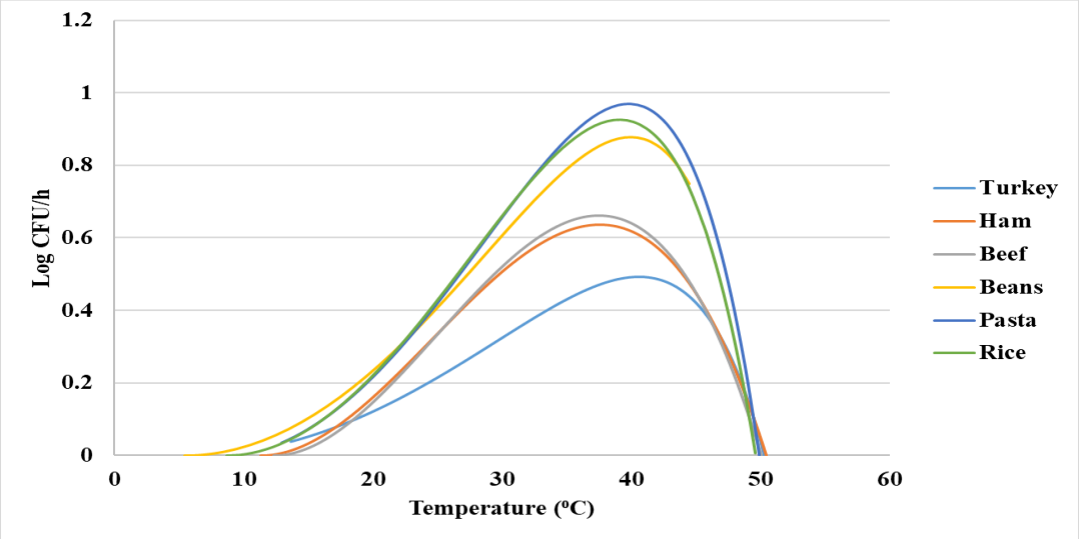


Figure 5.3: Validation of the developed predictive model for *B. cereus* in roast ham following two sinusoidal temperature profiles (Low sinusoidal temperature profile: 5-15ºC and High sinusoidal temperature profile: 10-40ºC).

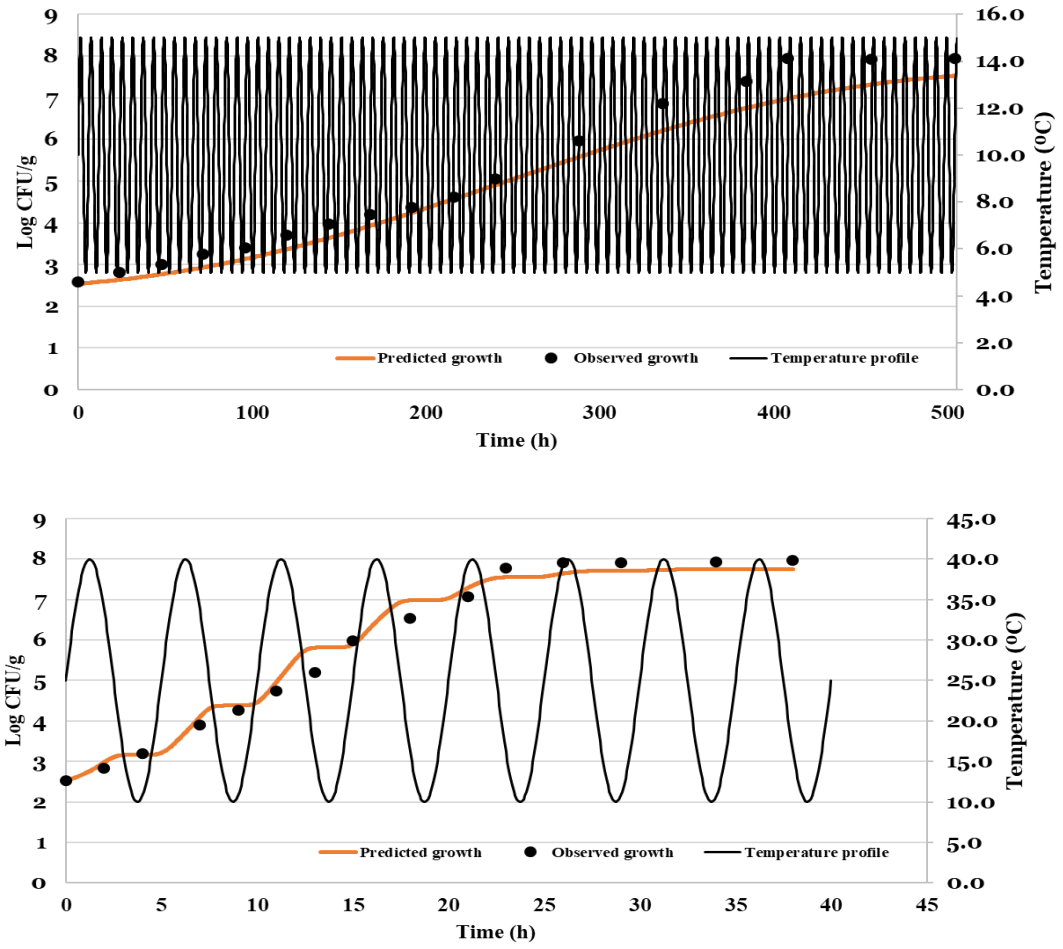
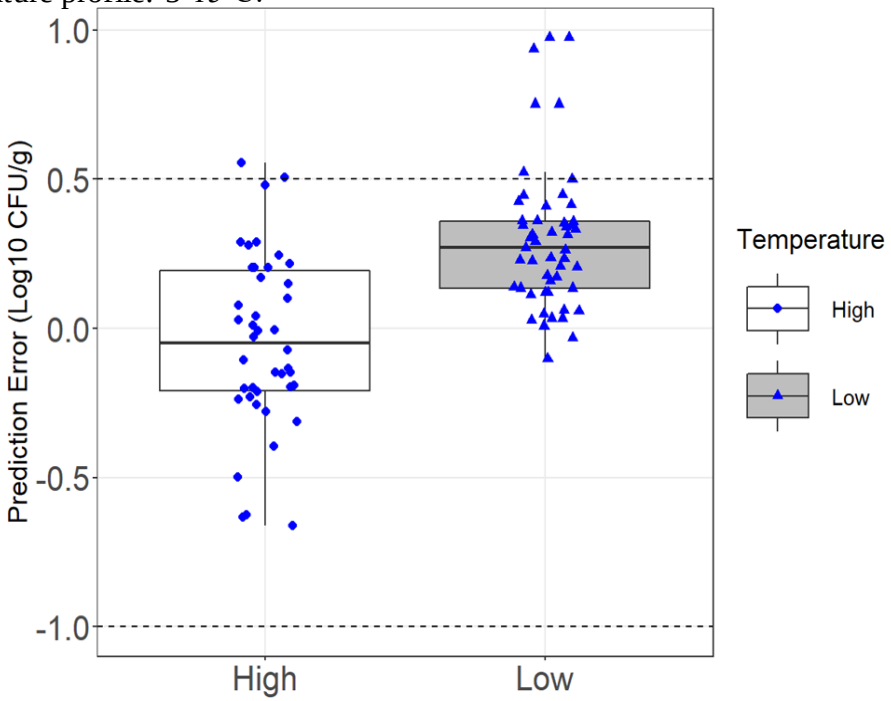


Figure 5.4: Acceptable Prediction Zone (APZ) analysis for the validation experiments - Left boxplot: High temperature profile: 10-40ºC; Right boxplot: Low temperature profile: 5-15ºC.



CHAPTER 6

CONCLUSIONS

Meat products, including turkey, beef, and ham have been implicated in numerous outbreaks with *B. cereus*. Contamination of raw materials and inadequate cooling or storage temperatures contribute to the presence of *B. cereus* in cooked foods. Existing predictive models for *B. cereus* growth have limitations, and dynamic models specific to meat products are needed. The dynamic models, including Baranyi, Ratkowsky, and tertiary models, performed exceptionally well, with all the goodness-of-fit parameters falling within acceptable ranges. This achievement encourages regulatory agencies and food processors to utilize these models to predict the growth of *B. cereus* in delicious ready-to-eat (RTE) cooked beef under various storage and temperature conditions, ensuring food safety. These models also aid in determining appropriate cooling rates to prevent the germination and spread of *B. cereus* spores. The integration of predictive models into extensive databases empowers industries and organizations to establish a strong fortress of food safety and preservation. The next generation of models holds the promise of advancing safety assurance, shelf-life control, hazard analysis procedures (HACCP), and other domains, ultimately eradicating the threat of foodborne illnesses caused by *B. cereus*.

CHAPTER 7

FUTURE RESEARCH

Further research can be conducted to observe the growth of *Bacillus cereus* in processed turkey, beef, and ham supplemented with various combinations of external conditions like pH, antimicrobials, and water activity levels to further evaluate the safety of these products against pathogen activity. Additionally, the effects of other non-pathogenic foodborne bacteria on the growth of *Bacillus cereus* could also be studied to determine if non-pathogenic foodborne bacteria compete with *Bacillus cereus* for nutrients and thus help diminish its growth. The general comparison of *B. cereus* activity in processed turkey, beef, and ham, as observed in the validation profiles developed for the current products in this study, suggests the need for further exploration of separate dynamic modeling approaches for various meat products. This could aid in the successful implementation of predictive models for meat processors and regulatory agencies to ensure microbiological safety of food to prevent foodborne illnesses caused by *B. cereus.*