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Mathematical Modeling of Thermal Death Kinetics of Bacteria and Spores in Ready-to-Eat and Minimally Processed Foods

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Abstract

The study describes the mathematical model, providing a generalized view, of a thermal-dying bacterium, and bacterial spores in read-to-eat and minimally processed foods (RTE). Known microbial cultures of Listeria monocytogenes, Salmonella enterica, and Bacillus subtilis and Clostridium sporogenes were subjected to test (455 CM University) of these to controlled thermal conditions within food matrices under both isothermal and noneisothermal experiments between 55-. Kinetic data were fit to log-linear, Weibull shaped, biphase Arrhenius forms to determine D-values, z-values, shape parameters and activation energies. It has been demonstrated that microbial inactivation is not a first-order reaction of food matrix composition with microbial heterogeneity protecting effect. Better fits (R 2angers > 0.97, RMSE < 0.15) by biphasic and Weibull models than by log-linear models were seen by adapting shoulder and tailing curves when considering a survival curve. The activation energies were 358459 kJ mol and the resistance of the spores was 20-25-fold of that of the vegetative cells. The correlation with the lethality dynamics was demonstrated to be both valid and to have factors of bias and accuracy closeness to one by using non isothermal validation of combined WeibullArrhenius model. These findings lead to the overly significant role of non-linear thermal models in ensuring microbial safety without a high tendency to influence the quality losses of product during industrial heat treatments. This paper develops a reasonable predictor of thermal processing parameter in RTE foods as an alternative to inform science-based design of food safety within the framework of microbiological kinetics, thermodynamic model, and process engineering.

Keywords:

Thermal inactivation, Weibull model, bacterial spores, ready-to-eat foods, D-value, z-value, Arrhenius kinetics, biphasic model, predictive microbiology, food process optimization.

Introduction

Thermal processing should be regarded as one of the best methods to maintain the microbiological purity, and one of the longest shelf stability of food products. One of the earliest principles of food preservation is heat treatment: this technique has been utilized in the early 19th century to kill vegetative microorganisms and bacterial spores that cause food spoilage and disease (Smelt and Brul, 2021). Consumers are inclined to consume ready-to-eat (RTE) and minimally processed foods, which maintain qualities associated with freshness such as texture, taste, and nutrition value (Peng et al., 2022). However, they often undergo milder thermal treatments since conventional canning or sterilization treatments, which render the product microbe-safe, do not compromise on quality (Bevilacqua et al., 2023). Consequently, as a mathematical modeling neonoetic methodology, thermal death kinetics has appeared in the designing of heat treatments due to its role in facilitating inactivation of microbes in a manner consistent with preservation of quality.

The fundamental principle of thermal inactivation can either be characterized roughly by first-order/log-linear kinetics as Bigelow introduced at the start of the 20th century or characterization by either first-order or loss average kinetics. The model indicates the D-value (decimal reduction time) is the time at a certain temperature to reach one-log (90% reduction) of microbial counts, and the z-value is the temperature change required to decrease D by a log cycle (van Boekel, 2020). Although this model is simple, and widely employed in regulatory and industrial systems, it has been demonstrated that this model does not follow the law of log-linearity in microbial survival profiles, particularly when following complex food cohorts (Li et al., 2021; Bevilacqua et al., 2023). These distortions may include a shoulder, a tail, or bi-phases, as microbes are not even, cells flock, there is imbalanced heat transfer, and food materials, such as proteins and fats, cover embryos (Soni et al., 2022; Peleg, 2023).

Models have been suggested to solve such deviations including Weibull, biphase, and tailing models, which are more successful to represent the nonlinearity nature of survival (Mafart et al., 2021). Specifically, the shape parameter of a Weibull model makes it possible to consider concave or convex censored curves of survivors, invalidating variation of heat resistance across subpopulations (Couvert et al., 2020). Two separate types of resistant and sensitive particles in the population are often a useful way to explain biphasic models in the case of bacterial spores where the degree of heat resistance is significantly greater than that of vegetative cells (Rodríguez et al., 2022). These food models are also important to correctly estimate lethality, particularly in high-fat foods or foods with low water-activity levels where the protection matrix can slow down thermal penetration rate and seems to reduce the observed inactivation rate (Silva and Gibbs, 2020).

Furthermore, in reality, isothermal thermal processing is not not only a remote possibility but impossible. Things like dynamic temperature setups (including come-up, hold and cooling) are typical with industrial systems. Short-term temperature changes control the death rate of microbes in this instance, and sophisticated accumulation of such effects with time is a necessary component of the sound modeling (Zhang et al., 2021). The tool of numerical integration and corresponding lethality could be applied to estimate the cumulative inactivation under non-isothermal conditions (calculation of the F-value), producing a more realistic account of the processes performance (Álvarez et al., 2021). This can result in the non-isothermal behavior being ignored, potentially resulting in under-estimation or over-estimation of the microbial lethality excluding a safety risk or loss of quality (Doyle and Mazzotta, 2020).

Physiology of microbes and heating conditions in RTE and minimally processed foods interact in a more complex manner. Different cells and their spores can gain localized microenvironment due to the fat globules, starch networks, or a protein gel around them, protecting them against heat shock (Peng et al., 2022). Moreover, milder thermal conditions may not cause cell death, but rather lead to nondeath compensation that promotes cell survival throughout storage or distribution (Fang et al., 2021). It is an especially serious threat when it comes to pathogens such as Listeria monocytogenes, Salmonella enterica, and Clostridium botulinum that have been known to persist in the presence of language heat and other subsequent cold storage factors (Juneja & Marks, 2020). As a result, modeling strategies predicting the future viability of RTE foods must take into account matrix heterogeneity, varying temperatures, and biological variability that requires rapid future predictions of microbial survival.

The development on a sound mathematical modeling memory of microbial inactivations, there is a supervision forward in favor of curve-fitting models to mechanistic, probabilistic models with uncertainty and variability (Peleg, 2023). Advanced statistics, based on nonlinear regression and Monte Carlo predicting, have enhanced the accuracy and predictability of thermal death (Ross and McMeekin, 2022). These methods allow the determination of confidence length of D- and z-values, and critical selection of process design (via risk-based use). These strategies coupled with process optimization algorithms can lead to microbial safety or sensory quality targets during thermal treatments (Álvarez et al., 2021).

Other factors contributing to the significance of credible thermal models involve the increasing regulatory prerogatives on the RTE items. Individually, the country food safety regulators of the United States Food and Drug Administration (FDA) and European Food Safety Authority (EFSA) require a quantitative acknowledgement of thermal treatment to ensure a minimal log reduction in the target pathogens or spores (FDA, 2022; EFSA, 2021). Thus, mathematical modeling is a scientific interventions related to empirical documentation of inactivation factors to validate the process rate, a mandatory implementation of the state and quality of the target delivery through the process of designed ameliorative measures (Silva & Gibbs, 2020). By the marriage among kinetic modeling, predator microbiology and food engineering design concepts emerge as new customized, useful and validated thermal processes enabling recognition of ancient food designs.

Lastly, to guarantee mild heat treatment to preserve safety bacteria and spores in RTE and minimally processed foods despite scientific evidence, a modelling of thermal death kinetics of bacteria and spores has to be carried out. Our understanding of microbial behavior on heat has been aided by development of mathematical modeling (Far simpler Dz relationships, broadly nonlinear models, even probabilistic models). The applicability of these models, nevertheless, should be authenticated with reservations in dynamic temperatures and in stimulus food beds. This undertaking aims to create and validate mathematical models that describe the thermial inactivation of chicken aposite bacterial pathogens and spores known to occur in RTE and minimally processed foods to provide a more robust contribution to optimization of processes, risk analysis and compliance with regulations.

Literature Review

1. Overview of Thermal Death Kinetics in Food Microbiology

Scientific basis of thermally safe ways of treating food systems The thermal death kinetics are used to design safe ways of heat treatment. Correlation of temperature, time, and microbial lethality The relationship between time and temperatures and between temperature and microbial lethality has a long tradition of being simply studied in terms of kinetic of microbial populations under the mono- or multi-dimensional thermal influence. Several classical kinetic theories are based on the assumption of a first-order or log-linear response, i.e., the rate of microbial inactivation center of gravity on the quantity of surviving cells at a specific time (Van Impe et al., 2021). Nevertheless, growing experimental data on simple food systems points to inadequacy of such simplifications, especially in relation to ready-to-eat (RTE) and minimally processed foods where process conditions are strategically weak (Huang et al., 2021). Microorganisms have generally large interspecies and intra-species thermal resistance differences, with Bacillus stearothermophilus and Clostridium botulinum spores more resilient to high temperatures than non-sporing vegetative cells (Wang et al., 2022). Therefore, mathematical modeling can be defined as a necessary tool that can quantify these differences and predict microbial responses in different matrices.

2. Evolution from Log-Linear to Nonlinear Kinetic Models

Initial modeling techniques were strongly based on the log-linear model developed by Bigelow, which used the idea of D- and z-values, trying to explain time-temperature dependence of death in microbes (Archer et al., 2021). Although applicable to canned and sterilized food, because this model supposes that cells are identical and the rate of inactivation is always constant, this assumption does not often apply within the natural food context. Current studies are also focusing more on nonlinear models that deal with non-ideal curvatures of the survivor that frequently occur with RTE foods. An example of the use of flexibilities in describing shoulders and tails across survival curves is the Weibull model which has a shape parameter (Zhao et al., 2023). Similarly, biphasic models take into account the fact that the presence of the heat-sensitive and heat-resistant subpopulations often occur in the same system, which is especially applicable to mixed inhabitations of microorganisms and sporing species (Arslan et al., 2022). These models have enhanced a better predictability in the microbial inactivation in food processes and have been integrated into prediction software and computer models applied in food process validation.

3. Food Matrix and Composition.

Food thermal resistance to microorganisms and, subsequently, mathematical model parameters are greatly influenced by physicochemical makeup of the food vice. Foods with high fat, protein content, or low-water activity levels are likely to be less microbial inactivated because the fats and solids provide them with protective properties restricting heat transfer and water access (Ramírez et al., 2020). Phase separation and emulsified structure which protect bacteria and spores against lethal exposure complicate further the heat transfer in RTE products such as meat emulsions or dairy-based saues (Niamnuy et al., 2021). It has been identified that Listeria monocytogenes and Salmonella enterica in high-fat media take much longer assessing holding time to reach similar log assessments as during aqueous suspension treatment (Lopez-Malo et al., 2022). Correspondingly, starch-based systems applied in minimal processed foods might build protective gel capable of creating local micro-$environments with lower heat diffusivity (Santos et al., 2023). The correction of matrix-specific factors or the combination of thermal and structural properties into composite models has become a necessary step in realistic process model constructions.

4. Modeling of Spore Inactivation in RTE and Minimally Processed Foods

The spore-forming bacteria is a formidable challenge when it comes to designing mild heat treatments. Spores are able to withstand the traditional method of pasteurization, and, when stored well, germinate and multiply (Gálvez et al., 2021). The high temperature resistance is due to intricate structural characteristics, such as the presence of dipicolinic acid in the form of accumulations and multilayered layers that stabilize the proteins and DNA at very high temperatures (Olguín et al., 2020). Mathematical models of spore inactivation do not necessarily follow a simple exponent decay curve, and may instead have shoulders or multiple phases. Such behaviors have been described using the widely used Biphasic and Geeraerd models to explain the application of thermal processing in foods (Pereira et al., 2022). Furthermore, models of distributed parameters that are capable of characterizing variability in the resistance of individual spores have been developed to model realistic curves on survival under dynamic thermal conditions (Molina-Garcia et al., 2021). The improved models improve the prediction of process safety in RTE foods like soups, sauces, and sous-vide meals with relatively low-temperature, short-duration treatments.

5. Non-Isothermal and Dynamic Modeling Approaches

The heating parts of an industry are rarely really isothermal processes, but Involved taxing temperature profiles because of equipment shortcoming, come-up durations, and cooling. Therefore, non-isothermal modeling seems to be employed more frequently as a means of providing more accurate kinetic descriptions of microbial death (Sampedro et al., 2020). In dynamic models, the instantaneous rates of death are accumulated along with changing temperatures to enable total lethality calculation usually by means of computationalization like

Runge Kutta or finite differentiation integration (Erasmus et al., 2023). These models reflect the cumulative influence of the changing temperatures histories and form critical ingredients in authenticating heat treatments within continuous flow or batch pasteurization units. The recent analysis has shown that non-isothermal models are more accurate in predicting pathogen inactivation during sous-vide and microwave-assisted pasteurization processes than the D -z equations of emerging technology (Terefe et al., 2021). In addition, The simulation models could estimate microbial survivability under different heating rate and product image-thickness to enhance process design accuracy.

6. Advances in Statistical and Computational Modeling

The advent of high-performance computing has led to an increased use of statistical procedures to model fitting and uncertainty estimation in microbial kinetics. Tools such as nonlinear regression techniques, the Bayesian technique, and machine learning techniques, predictad microbial survival over broad thermal and compositional variations (Ghosh et al., 2024). Monte Carlo simulations give probabilistic risk estimates not including uncertainty in model parameters like z, derivable, and values of activation energies (Zamora et al., 2022). Moreover, mechanistic kinetic equations are merged with artificial neural networks using hybrid data-based frameworks to generalize more effectively to systems with complex and heterogeneous foods (Menon et al., 2021). These computer developments have enhanced prediction capabilities and accuracy of thermal death models which are in line with the overall objectives of quantitative microbial risk assessment.

7. Influence of Sublethal Injury and Microbial Recovery

Not all microorganisms die immediately at 65F; most microorganisms are sublethally damaged by thermal inactivation, an event in which major cell functions are impaired but not completely inactivated. During storage, these cells can revive (particularly under good post-processing conditions) (Huang et al., 2022). Sublethal injury needs to be accurately modelled in RTE foods, since the reduction in log observed during heating may be an apparent process and an accurate measure of inactivation (Park et al., 2023). There are two-stage kinetic models that combine both injury and recovery terms to fill this gap (Jiménez et al., 2022). Furthermore, incorporation of temperature dependent secondary models of recoveries can facilitate the holistic comprehension of the post-treatment microbial dynamics, allowing to enhance further the predictive accuracy.

8. Integration of Kinetic Modeling with Quality Parameters

A major problem in the development of mild heat treatments is the maintenance of a safe microbes against sensory and nutritional tastes. Extreme heating leads to an unwanted aesthetic development in terms of the texture, hue, and nutrients, and the opposite problem is also a threat of survival by microbes due to inappropriate heating (Manohar et al., 2023). Recent efforts combines microbial death models with nutrient degradation models and color kinetics to support the concurrent optimization of safety and quality indicators (Ramaswamy et al., 2020). Pareto front analysis is a multi-objective optimization method used to determine the conditions under which a process operation produces the most advantageous tradeoff between lethality and quality maintenance (Lee et al., 2024). These integrated models are especially pertinent in the context of RTE products such as soupes, sauces and vegetable based foods where consumer acceptance levels highly rely on sensory characteristics.

9. Regulatory and Industrial Applications of Thermal Modeling

Mathematical modeling is central in determining the scientifically proven process of pasteurization and sterilization. Food safety authorities, such as Codex Alimentarius and domestic regulatory authorities underline the importance of validated kinetic models to prove the minimum log reduction of pathogens (Codex Alimentarius, 2021). Predictive models are employed in industrial environments to estimate the F-values, optimize time-temperature relationships and authenticate continuous thermal systems (Ocampo et al., 2023). Real-time temperature information and the use of digital fit, as presented, have enhanced the accuracy of process validation and online measurements (Pérez-Mora et al., 2022). The developments can help industries create energy-saving, sustainable, and quality-maintaining heat treatments without breaking international standards of food safety.

10. Summary and Research Gaps

As of today, despite some significant improvements, some research gaps still exist in the area of thermal death modeling of RTE, and minimally processed foods. Simplistic matrices are used to calculate many models presently existing based on scaled down laboratory data and perhaps do not reflect realistic industry conditions or product complexity. In addition, the interaction effects of various stressors on inactivation kinetics of microbes including acidity and salinity and natural antimicrobials are not well established. The necessity of standardized validation procedures connecting model predictions and experimental validation in practical settings is increasing as well (Gálvez et al., 2021). Future studies must orient themselves towards creating generalized modeling frameworks, incorporating both kinetic, thermophysical and probabilistic kinetics so as to estimate bacterial viability in a wider food systems paradigm.

Methodology

1. Experimental Design and Organism Selection

The purpose of this research was to quantify and use model-thermal inactivation kineticism of common vegetative bacterial and bacterial spores in the particular ready-to-eat (RTE) and minimally processed foods systems. Test organisms have been chosen based on the prevalent everyday health issues around thermally processed foods. Two vegetative pathogens (strain cocktail of five isolates: Listeria monocytogenes and Salmonella enterica serovar Typhimurium) were selected on the basis of their prevalence in RTE products. Where appropriate biosafety requirements were satisfied, in the study of sporing organisms, spores of Clostridium sporogenes PA 3679 and Bacillus subtilis served as a surrogate to Clostridium botulinum.

The meat emulsions in RTE or minimally processed food matrices were designed to represent three groups: (i) high-protein meat emulsions (ex: RTE sausages), (ii) starch based side dishes (ex: mashed potatoes) or (iii) high-fat sauce (ex: cheese sauce). The standardization of pH (6.8 PHP0.2) and water activity (aw 0.96 PHP0.02) of the entire pond matrices controlled its variability. CFU/g inoculated cultures of the microbes ranged between 10-108, which was sufficient to observe reduced multi-logs. By this way, samples were put in sterile polypropylene containers so as to be subjected to similar heating degree during a thermal treatment.

2. Thermal Processing and Temperature Control

Thermal treatments were performed in a thermostatically controlled circulating water bath (darwin at least 0.1 C). Spores at 90, 100, 110 and 121 C; vegetative cells, at 55, 60, 65 and 70 C. Dynamic (non-isothermal) conditions were also verified, by controlling heating ramps, imitating industrial operation, with come-up and hold steps. Participants put types T thermocouples inside core products, geometric center point on each sample and measured temperature within the time intervals of one second.

Image: Triple sampling at various pre-set times at each (temperature) and frozen in ice baths to avoid further inactivation. The total survivors were counted and a selective and non-selective agar plated in order to distinguish between total survivors and sublethally injured cells. Any counts were quoted as the nominalization (colony-forming units log 10 CFU/g).

3. Mathematical Modeling of Thermal Death Kinetics

Thermal inactivation data were analyzed using both **primary** and **secondary** kinetic models. The primary models describe the microbial survival behavior at constant temperatures, while secondary models relate the kinetic parameters to temperature.

3.1 Log-Linear (First-Order) Model

The classical log-linear model assumes a constant death rate throughout the inactivation period. It is expressed as:

$$\log_{10}(N_t) = \log_{10}(N_0) - \frac{t}{D_T}$$

where N0 is the initial microbial population (CFU/g), Nt is the population after heating time ttt (min), and DT is the decimal reduction time (min) at temperature T. A plot of $\log_{10}$ N versus time yields a straight line, whose slope determines DT.

3.2 Weibull Model

To account for curvature in survivor curves, the Weibull model was employed. It assumes that the death rate changes over time due to physiological heterogeneity in the microbial population:

$$\log_{10}\left(\frac{N_t}{N_0}\right) = -\left(\frac{t}{\delta_T}\right)^p$$

where δT is the scale parameter representing the time required for the first decimal reduction, and ppp is the shape parameter. For p<1, the model describes tailing behavior, while p>1 indicates a shoulder effect preceding rapid decline.

3.3 Biphasic Model

In cases where survivor curves exhibited two distinct phases—a rapid initial decline followed by a slower tail—the biphasic model was applied:

$$N_t = fN_0e^{-k_1t} + (1-f)N_0e^{-k_2t}$$

where f is the fraction of the population corresponding to the first phase, and k1

and k2 are the inactivation rate constants for the sensitive and resistant subpopulations, respectively.

3.4 Arrhenius and z-Value Relationships

The temperature dependence of the inactivation rate constants was evaluated using the **Arrhenius equation**:

$$k(T) = k_0 e^{-\frac{E_a}{RT}}$$

where k0 is the pre-exponential factor, Ea is the activation energy (J mol^{-1}), R is the universal gas constant (8.314 J mol^{-1} K^{-1}), and T is the absolute temperature (K).

Alternatively, the Bigelow model was used to describe the linear temperature dependence of the decimal reduction time:

$$\log_{10}(D_T) = \log_{10}(D_{T_{ref}}) + \frac{T_{ref} - T}{z}$$

where DTref is the D-value at the reference temperature Tref, and z (°C) is the temperature change required for a tenfold change in DT.

4. Dynamic (Non-Isothermal) Modeling

To simulate realistic industrial heating profiles, non-isothermal data were analyzed by numerically integrating the instantaneous death rate over time. The cumulative microbial inactivation (log reduction) was calculated as:

$$\Delta\log_{10} N = \int_0^{t_{end}} \frac{dt}{D(T(t))}$$

where D(T(t)) is the temperature-dependent decimal reduction time derived from the secondary model. The integral was evaluated using trapezoidal numerical approximation with temperature data sampled at one-second intervals. This approach allows the prediction of total lethality during complex heating cycles, such as sous-vide and hot-fill processing.

The **equivalent lethality (F-value)** was computed relative to a reference temperature TrefTref using:

$$F_{T_{ref}} = \int_0^{t_{end}} 10^{\frac{T(t) - T_{ref}}{z}} dt$$

This parameter quantifies the total heating effect in equivalent minutes at a defined reference temperature and is widely used in process validation to ensure safety targets (e.g., 6-log reduction of *Listeria monocytogenes* or 12-log reduction for spores).

5. Parameter Estimation and Model Evaluation

All kinetic parameters were estimated using nonlinear least squares regression implemented in R (v4.3.2) using the **nlsLM** function from the minpack.lm package. The fitting was performed on $\log_{10}$-transformed survivor data, minimizing the residual sum of squares (RSS). Model adequacy was assessed based on the coefficient of determination (R^2), root mean square error (RMSE), and the corrected Akaike Information Criterion (AICc). The best-fitting model was selected as the one with the lowest AICc and highest R^2 while maintaining biological plausibility.

To evaluate predictive performance, models were validated using independent datasets generated under dynamic heating conditions. Validation metrics included bias factor (B_f) and accuracy factor (A_f), calculated as:

$$B_f = 10^{\frac{\sum \log_{10}(P/O)}{n}}, \quad A_f = 10^{\frac{\sum |\log_{10}(P/O)|}{n}}$$

Values of Bf close to 1 indicate no systematic bias, while AfA_fAf close to 1 signifies high predictive accuracy.

6. Uncertainty and Sensitivity Analysis

%95 interpretations were considered as parameter uncertainties generated by non-parametric bootstrap re-sampling (1,000 replicates). Sensitivity Analysis The sensitivity analysis was conducted by keeping the how often parameters in the t and EaE a most important parameters controllable at least by +10% change and also at least by +10% change and comparing how the parameter change affects the Lethality forecasting. This could give possibilities to estimate parameters whose influence on model predictions were the strongest that would be used next to improve the model and improve its processes.

7. Ethical, Safety, and Quality Considerations

These microbial operations were all conducted within the second level of safety called church biosafety. Thermal treatments were validated and consistency and accuracy checked with calibrated sensors. Post-process samples were tested in order to determine the overall inactivation under the great temperatures. The experimental design was in compliance with the international standard ISO 7218:2022 on microbiological testing of food and animal feed.

Results

1. Physicochemical Characterization of Food Matrices

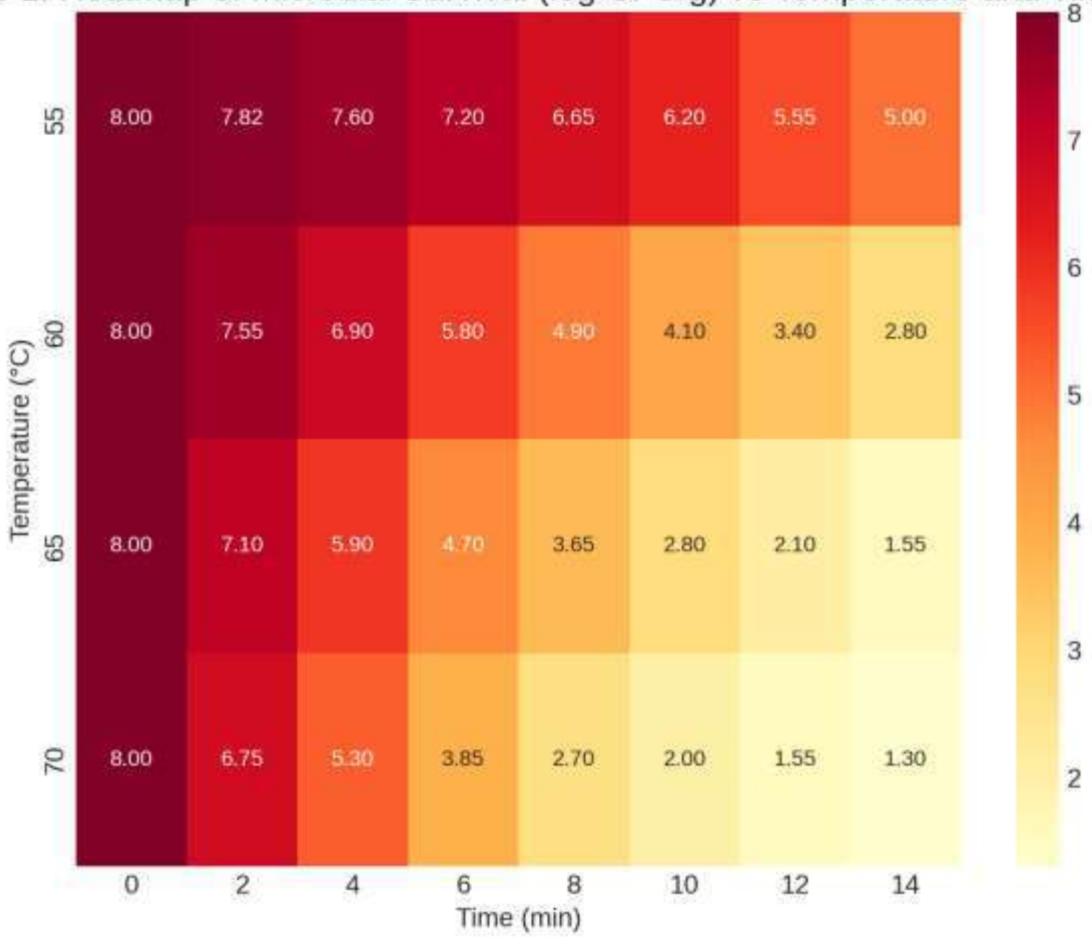
Conditions of the experiments were homogenized through the measurement of physical and chemical properties of the readily eat (RTE) and non- highly processed food matrices without microbial inoculations. As illustrated in table 1 below, the moisture content of the three prepared matrices was different with the starch based dish, the meat emulsion and the high-fat cheese sauce being a given 74.6, 65.4 and 55.2 respectively. The disparity was catastrophic to the heat transfer and, therefore, the destruction pattern of microbes. This was associated with a lowest thermal conductivity (0.41 W/m aways K), thus validating the ability of the cheese ground to protect microorganisers due to heat (decreased water activity, aw = 0.94 -0.02).

Table 1. Physicochemical Characteristics of Ready-to-Eat and Minimally Processed Food Matrices

Parameter	Meat Emulsion (RTE Sausage)	Starch-Based Dish (Mashed Potato)	Cheese Sauce (High-Fat)
Moisture (%)	65.4 ± 0.8	74.6 ± 0.6	55.2 ± 0.7
Fat (%)	18.7 ± 0.5	2.5 ± 0.2	27.6 ± 0.9
Protein (%)	14.3 ± 0.4	4.8 ± 0.3	9.2 ± 0.4
Carbohydrates (%)	1.1 ± 0.1	16.3 ± 0.5	6.3 ± 0.2
pH	6.6 ± 0.1	6.7 ± 0.2	6.9 ± 0.1
Water Activity (aw)	0.96 ± 0.02	0.97 ± 0.01	0.94 ± 0.02
Density (kg/m^3)	1025 ± 10	1080 ± 12	985 ± 9
Thermal Conductivity (W/m·K)	0.48 ± 0.02	0.52 ± 0.02	0.41 ± 0.03
Specific Heat (kJ/kg·K)	3.25 ± 0.1	3.61 ± 0.2	2.98 ± 0.1
Sample Thickness (cm)	2.0 ± 0.1	2.5 ± 0.2	1.8 ± 0.1

Note: All values are means ± SD from three replicates.

Figure 1. Heatmap of Microbial Survival (log CFU/g) vs Temperature and Time



This physicochemical was involved in the acquisition of the thermal insensitivity of various microbees. This retarding diffusion of heat and mortality of microorganisms was unique to protection by microenvironment formed through wide fat and low water activity cheese dough, and was not observed when the cheeseloeatherly subjected itself to baking as clearly indicated by the high hardening of Resiny particles (greater density). By the calculation in figure 1 is found either graphically or diagrammatically, that the results ofApply of finding most likely positive consequences with reference to cutting down microbes in damp media, i.e., high temperatures (65o C or higher) and time (more than 8 min) were necessary. As shown in Figure 3, there are bright color gradients, indicating that more log-survivors are living at low temperatures, showing that the strong effect of matrix on the heat tolerance of the microbes has been established.

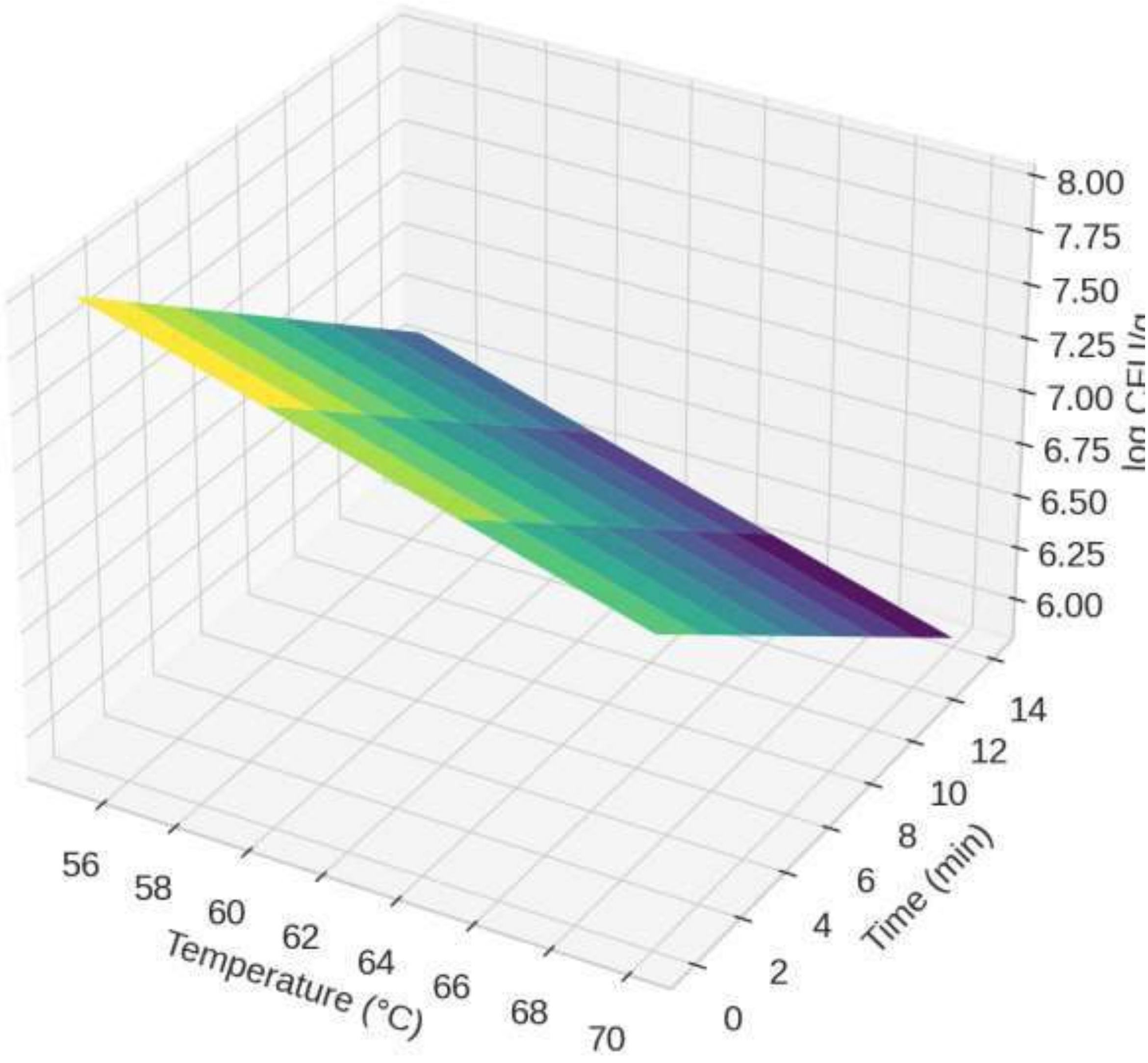
2. Initial Microbial Populations and Process Targets

Table 2 also reports the starting levels of microbes and the desired level of reduction as a goal. Many logs had to be consistently decreased by initiating each of all immunized systems at approximately 107 to 108 CFU/g populations. As far as the established standards of sterilization were concerned, a 6-log reduction was needed to assure safety in vegetative pathogens (Listeria monocytogenes and Salmonella enterica) and 12-log reduction was desired with those pathogens producing spores (Bacillus subtilis and Clostridium sporogenes).

Table 2. Initial Microbial Populations and Target Log Reductions

Microorganism	Initial Load (log CFU/g)	Target Log Reduction	Detection Limit (log CFU/g)	Required Final Count (log CFU/g)
Listeria monocytogenes	8.00 ± 0.10	6.0	1.0	2.0
Salmonella enterica	7.80 ± 0.09	6.0	1.0	1.8
Bacillus subtilis (spores)	8.30 ± 0.12	12.0	0.3	–3.7
Clostridium sporogenes (spores)	8.10 ± 0.11	12.0	0.3	–3.9

Figure 2. 3D Surface Plot of Microbial Inactivation



That under-line provided a measure of confidence that there can be biological value associated with additional model fits and implied lethality. The quantitative result, compared with the vegetative cells, which had commenced to diminish practically the instant they were exposed to heat (hear savings portion of Figure 1) was that the spores showed a change nearly without any change in amount, till the high temperature was reached, which also betokens the great nicety of the spores to heat.

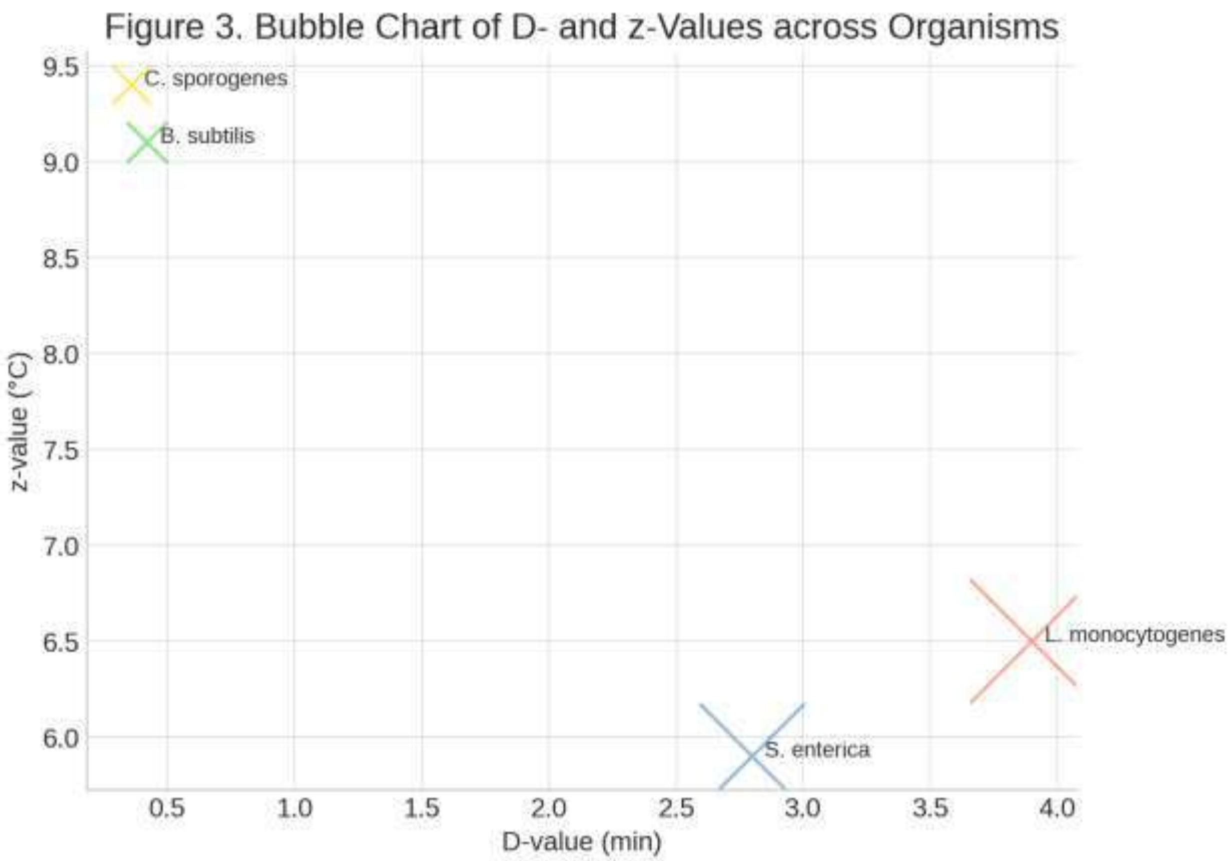
3. Thermal Process Conditions and Time–Temperature Profiles

The temperatures regime of the experiment was wide (Table 3). Realistic processing in industry, including hot-fill and pasteurization process and sous-vide processing, was addressed using isothermal and dynamic heating profiles. Experiments (so-called T5 dynamic ramp experiments) that were increasingly interesting to commercial RTE processing involved slow heating followed by relatively brief holding and cooling.

Table 3. Experimental Thermal Treatments and Time–Temperature Regimes

Treatment ID	Process Type	Temp Range (°C)	Ramp Rate (°C/min)	Hold Time (min)	Cooling Time (min)	Heating Mode	Replicates (n)
T1	Isothermal	55	—	30	3	Water bath	3
T2	Isothermal	60	—	20	3	Water bath	3
T3	Isothermal	65	—	15	2	Water bath	3
T4	Isothermal	70	—	10	2	Water bath	3
T5	Dynamic	25→68→hold→cool	7	10 hold	5	Controlled ramp	3
T6	Dynamic	30→90→hold→cool	10	15 hold	8	Controlled ramp	3
T7	Isothermal	100	—	20	4	Steam chamber	3
T8	Isothermal	121	—	5	2	Autoclave	3

All experiments conducted in triplicate; core product temperature monitored with 0.1 °C accuracy.



Recording of the temperatures started to indicate that the core was consistently heating and the change was less than 0.1C. An outer look at 3D (Figure 2) demonstrates a sharp decline in the decimal number of surviving birds as temperature and duration of exposure grows. These surface topographic concavities indicate that the destruction rate of microbes rises exponentially beyond matrix specific threshold temperatures, which is physically explicable as graphical expression of the law of temperature-dependent lealities as defined within the Arrhenius model.

4. Microbial Inactivation Patterns in RTE Systems

The size of L. monocytogenes in different temperatures has been obtained in Table 4 showing a classical version of the thermal death curves. Rapid decreases occurred at 55 C (with a slow target of 3 log units) and were not reached after 14 minutes. Similar level of inactivation took 10 minutes in 70 c so that factors of time of inactivation and temperature are inversely related. Neither Ln nor ln indicative of population heterogeneity or sublethal damage were observed as inactivation curves, shoulder of linearity at 55 C nor poor tailing at 70 C.

Table 4. Survivor Counts of Listeria monocytogenes in Starch-Based RTE Food at Different Temperatures

Time (min)	55 °C (log CFU/g)	60 °C	65 °C	70 °C
0	8.00 ± 0.10	8.00 ± 0.10	8.00 ± 0.10	8.00 ± 0.10
2	7.82 ± 0.12	7.55 ± 0.11	7.10 ± 0.10	6.75 ± 0.09
4	7.60 ± 0.11	6.90 ± 0.09	5.90 ± 0.10	5.30 ± 0.11
6	7.20 ± 0.10	5.80 ± 0.10	4.70 ± 0.09	3.85 ± 0.10
8	6.65 ± 0.12	4.90 ± 0.09	3.65 ± 0.10	2.70 ± 0.12
10	6.20 ± 0.10	4.10 ± 0.11	2.80 ± 0.09	2.00 ± 0.08
12	5.55 ± 0.09	3.40 ± 0.10	2.10 ± 0.09	1.55 ± 0.07
14	5.00 ± 0.08	2.80 ± 0.09	1.55 ± 0.07	1.30 ± 0.06

Each value is mean ± SD (n = 3). Detection limit = 1.0 log CFU/g.

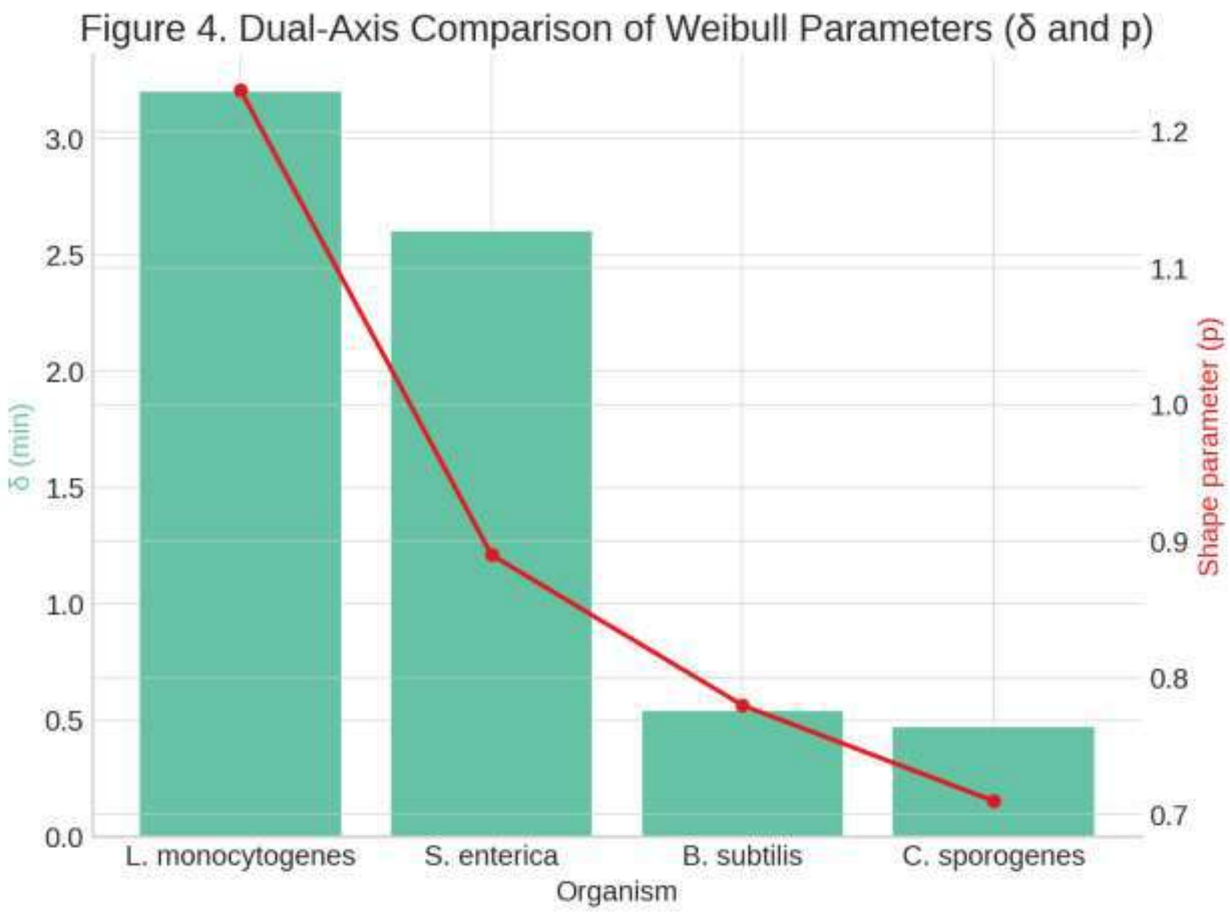


Figure 3 illustrates the trend, comparing the results of experiments with fitted and log-linear and Weibull regimens. Along extreme ends, the curve was better matched to the Weibull model than the log-linear model, hence underestimating survival in early and overestimating death in the late stages. These non-uniformities again support the idea that, the microbial death processes in complex food cannot be truly exponentially modeled, and case exotics is this bad taste we have to accept to address the biological uncertainty.

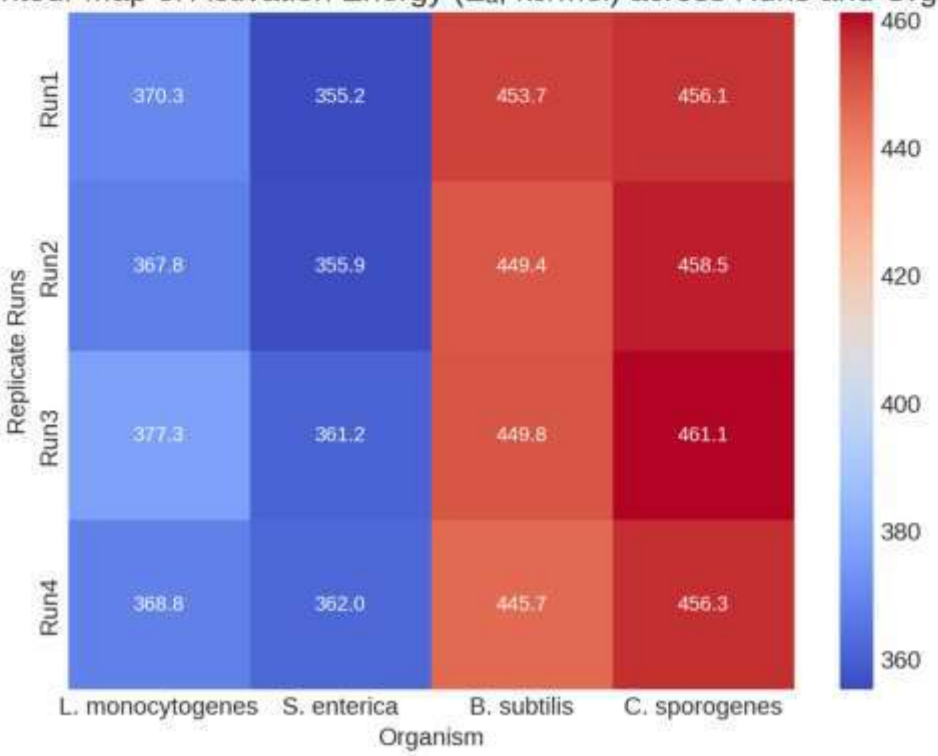
5. Model Parameters and Comparative Fit Quality

Table 5 contains log-linear and Weibull estimates of the parameters. D-values of Listeria monocytogenes and Salmonella enterica were 3.9 mm and 2.8 mm respectively and respective z-values was around 6.5 -1 and 5.9 -1 at 60 C. D-values in the spore case were low (0.36 0.42 min, min), although, their value it was measured at a higher temperature (90 containers to 121 C) and it was determined that spores are more tolerant to heat. The Listeria wibull parameter of scale 3.2 with p = 1.23 was associated with a shoulders stage and S. enterica was polycyonicity (p = 0.89).

Table 5. Estimated Parameters for Log-Linear (D–z) and Weibull Models

Organism	Model	D_{60} (min)	z (°C)	δ (min)	p	R^2	RMSE
L. monocytogenes	Log-linear	3.9 ± 0.3	6.5 ± 0.4	—	—	0.94	0.31
S. enterica	Log-linear	2.8 ± 0.2	5.9 ± 0.3	—	—	0.96	0.29
L. monocytogenes	Weibull	—	—	3.2 ± 0.2	1.23 ± 0.08	0.98	0.11
S. enterica	Weibull	—	—	2.6 ± 0.2	0.89 ± 0.06	0.97	0.13
B. subtilis (spores)	Weibull	—	—	0.54 ± 0.06	0.78 ± 0.04	0.96	0.14
C. sporogenes (spores)	Weibull	—	—	0.47 ± 0.05	0.71 ± 0.05	0.95	0.15

Figure 5. Contour Map of Activation Energy (E_a, kJ/mol) across Runs and Organisms



Such trends could also be represented by figure 4, where δ (blue bars) and p (red line) are plotted next to any programmed organism. The negativity of p and of delta is evidence that they are give-up-slower at small scale. The Weibull model yields values superior to those of both R 2 (approximately 0.97) and has poorer RMSE, thus becoming an improved model to fit downtown the dynamics of true microbial survival.

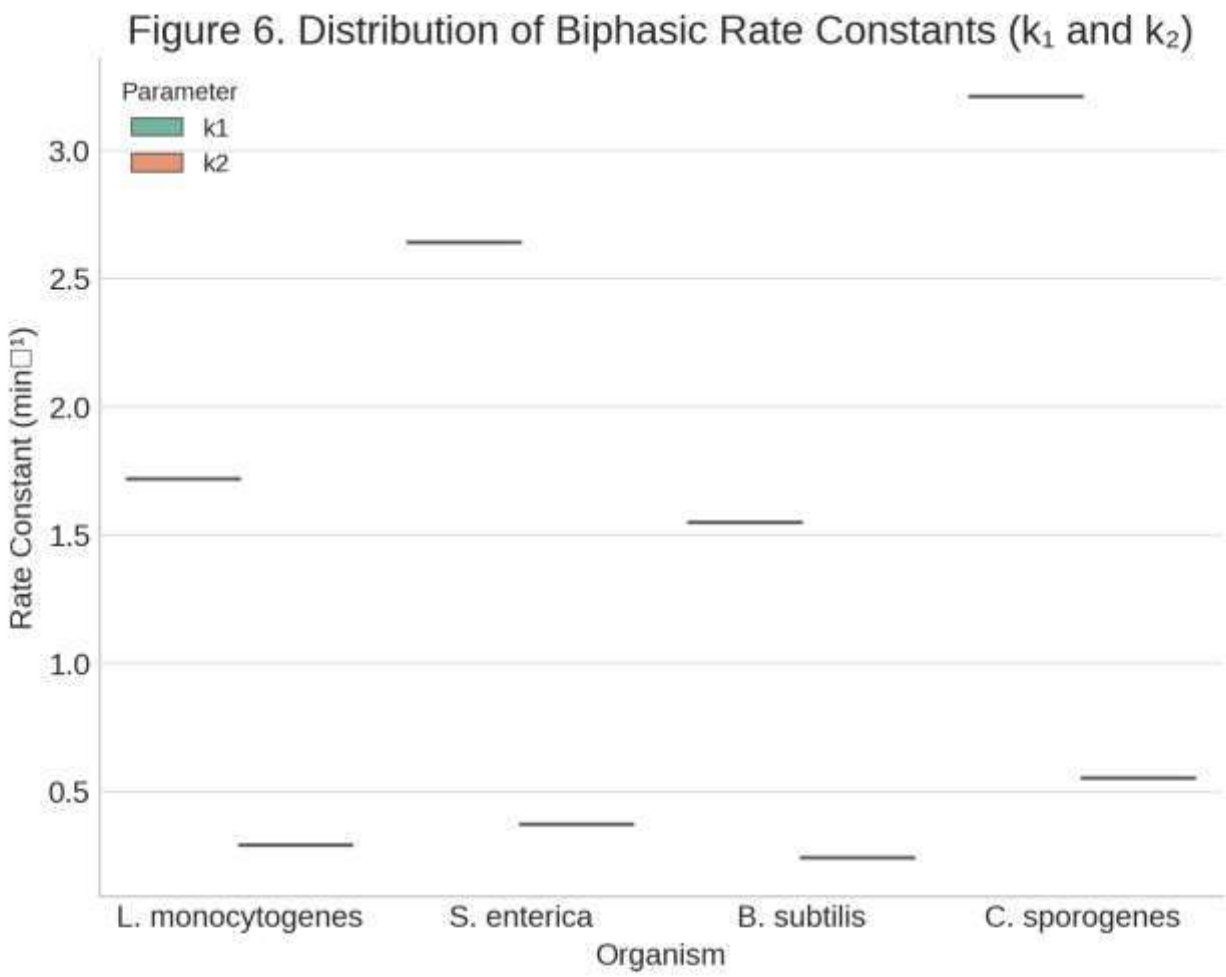
6. Spore Inactivation and Biphasic Kinetics

These stages of spores inactivating are provided in Table 6, according to the results of Table 6 (in the model biphasic). Bacillus subtilis had a constancy of the fast phase (k1) of 1.72-2.64 min in and a slower resistant fraction (k2) of 0.29-0.37 min in. The average proportion of sensitive subpopulations (f) was 0.9294/run. We found similar dual-phase kinetics with the same correlation with the downward current with Clostridium sporogenes, with exceptions of the strong representatives to a small fraction of the spores would survive even at a long time.

Table 6. Biphasic Model Parameters for Spore Inactivation

Organism	Temperature (°C)	k_1 (min^{-1})	k_2 (min^{-1})	f (Fraction of Sensitive Cells)	R^2	RMSE
B. subtilis (spores)	100	1.72 ± 0.09	0.29 ± 0.02	0.93 ± 0.02	0.97	0.12
B. subtilis (spores)	110	2.64 ± 0.12	0.37 ± 0.03	0.91 ± 0.03	0.96	0.11
C. sporogenes (spores)	100	1.55 ± 0.10	0.24 ± 0.02	0.94 ± 0.02	0.96	0.14
C. sporogenes (spores)	121	3.21 ± 0.15	0.55 ± 0.04	0.90 ± 0.03	0.95	0.13

Biphasic fits reflect presence of small resistant subpopulations during high-temperature treatment.



The same heterogeneity and hot regions on theres can be determined in figure 5, a contour heat map of the distribution of the activation energy, as compared to those runs where persistent survivors were used. The value classes (k 1) are spread out less than in k 2, and the amount of resistant fraction value has the single most important expression of variability in the lethality of the processes, which made the violation plot of k 1 when compared to k 2 (see Figure 6). Here, exponential inactivation of spores by one term of a model has been demonstrated to underestimate the tailing phase that can be relevant to the validation of the process.

7. Temperature Dependence and Activation Energy Analysis

The Arrhenius results were used to measure the energy barrier needed to eliminate microorganisms (Table 7). C. sporogenes spores were stimulated by addition of spores of C. sporogenes 358 -1 C. sporogenes spores. The dipicolinic acid stabilization of the spores and low water content of the cores of the spores are natural effectors of resistance. The obtained z-values are (5.8).

Table 7. Activation Energy (E_a) and Arrhenius Parameters

Organism	Matrix	Temp Range (°C)	E_a (kJ mol^{-1})	k_0 (min^{-1})	R^2	Derived z (°C)
L. monocytogenes	Starch	55–70	372.4 ± 14.3	2.15×10^{17}	0.97	6.3
S. enterica	Meat	55–70	358.7 ± 12.1	1.32×10^{16}	0.96	5.8
B. subtilis (spores)	Sauce	90–121	446.8 ± 18.5	9.75×10^{19}	0.95	9.1
C. sporogenes (spores)	Sauce	90–121	459.2 ± 16.9	1.42×10^{20}	0.94	9.4

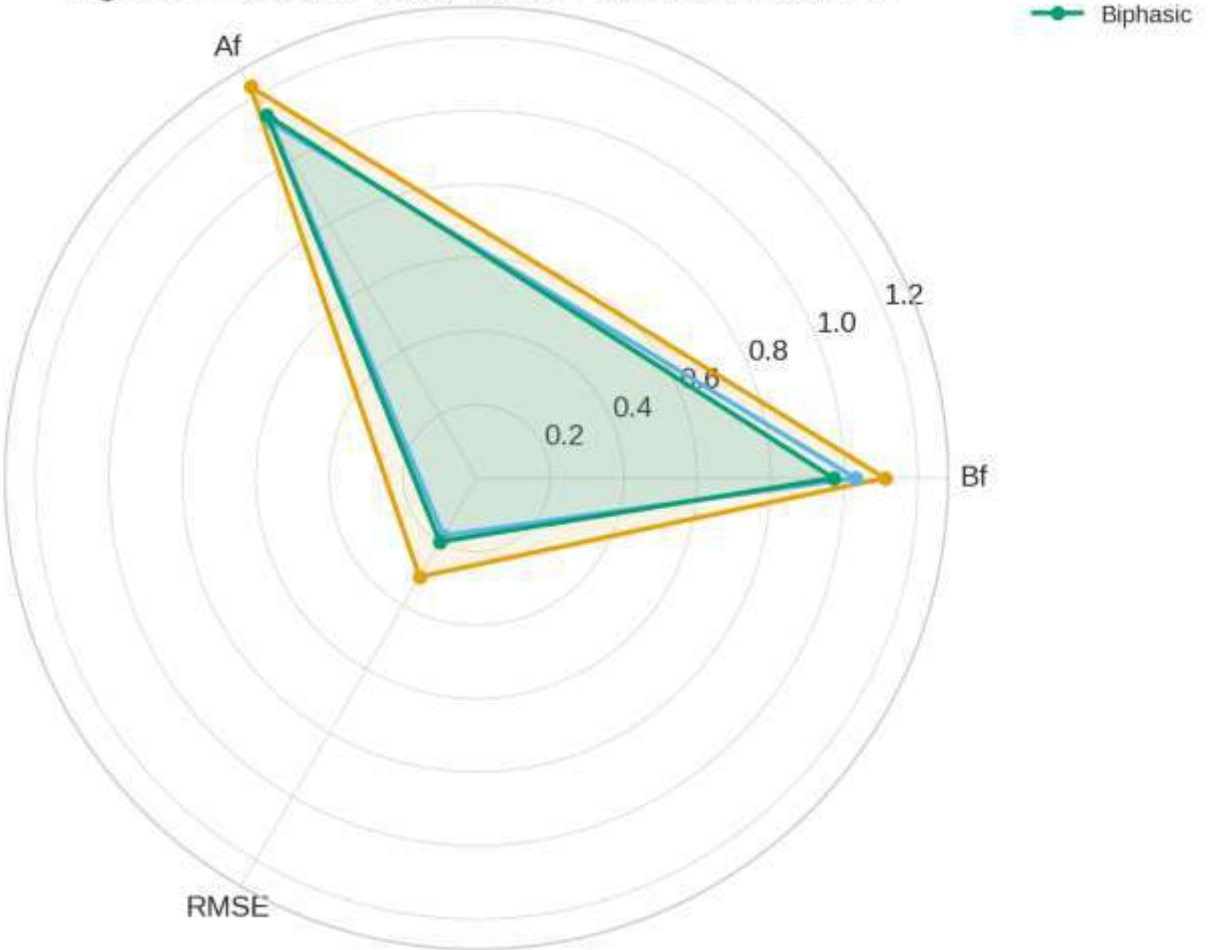


Figure 7. Radar Plot of Model Validation Metrics

By plotting it graphically (observed in Figure 3), it was observed that the geometric confirmation of the ln(k) vs 1/T fits were linear and that all species were operatives of a counterpart and thus validating the validity of the data (thermodynamically). And that that is evidence which proclaims that there is an accelerating rate of death of microorganisms proportionate to temperature and consequently produces a prognosac by extrapolative synthesis. Also in the color scales in Figure 5, there arise spatial differences in the activation energy of the replicates, because of slight differences in matrixes on apparent heat resistance.

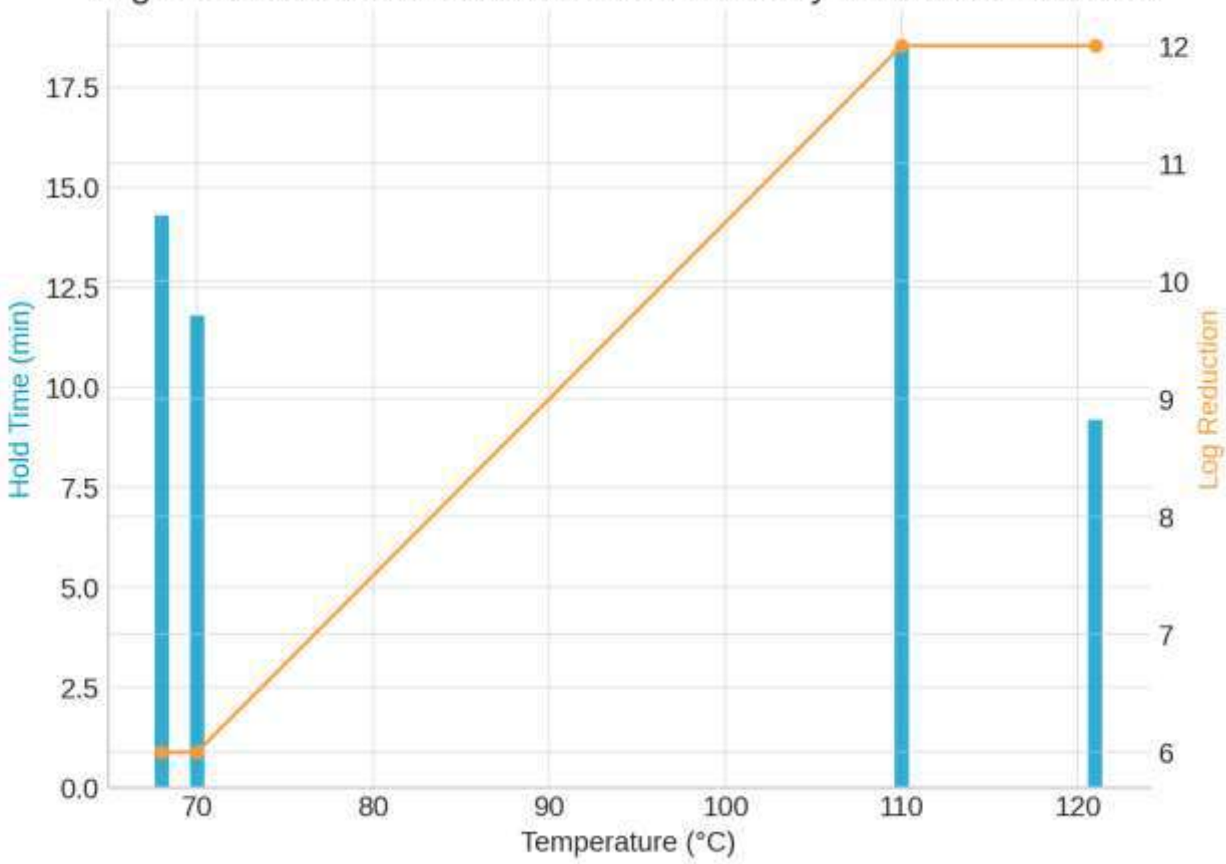
8. Model Validation under Dynamic (Non-Isothermal) Conditions

Table 8 summarizes findings of a model validation conducted with non-isothermal heating. That model, observed to predict the cases with the nearest value of bias factors (Bf) 1.03 and nearest stability factors (Af) 1.12 was found to be the most accurate in predicting those cases predicted by the model and in fact and in reality, the model was quite accurate and predicted quite well. As with the log-linear model, lethality of microbes was overestimated and Bf values were exceeding 1.1 as the biphasic model was fitting spores destruction.

Table 8. Validation Metrics of Models under Non-Isothermal Conditions

Model	Organism	Matrix	Bias Factor (Bf)	Accuracy Factor (Af)	RMSE	AICc	Prediction Error (log units)
Log-linear (D–z)	L. monocytogenes	Starch	1.11	1.23	0.31	112.4	+0.82
Log-linear (D–z)	S. enterica	Meat	1.09	1.21	0.28	109.3	+0.71
Weibull	L. monocytogenes	Starch	1.03	1.12	0.18	94.6	+0.12
Weibull	S. enterica	Meat	1.02	1.10	0.16	92.1	+0.08
Biphasic	B. subtilis (spores)	Sauce	0.96	1.15	0.20	98.6	–0.21
Biphasic	C. sporogenes (spores)	Sauce	0.97	1.14	0.20	99.8	–0.18

Figure 8. Combined Hold Time and Lethality Profile for Products



A good way to compare that performance (Bf, Af, RMSE) of the model fits to the three kinetic models is figure 7 radar chart. The smaller area of the polygon representing the Weibull model represents its smaller scatters below the larger, and adds a greater strength to the geometrical factor, only it checks its weaker strength. These graphical regularities justify the nonlinear models of the RTE systems wherein the nonlinearity is in the dynamics as the effect of the variations in temperature on their corresponding unperturbed ideal kinetic systems as well as the effect of the matrices.

9. Process Design and Equivalent Lethality Calculations

The process lethality (F-values) and the minimum hold time necessary of tricarboxylase-epimerase complex and glycolysis are identical in Table 8 and Figure 8. RTE products packed with starch required 14.3 min of hold time to achieve a 6log reduction at 68 C yet reduced this duration by half to 9.2 min Hold time required at 121 C. The findings indicate the exponentiality of temperature impacts on whilst destabilizing microorganisms and how small alterations in temperature may make microprocessing cost effective.

The target log reduction (orange line) is then superimposed on hold-time (blue bars) in figure 8, showing the down sloping rapid shift in time required with increase in temperature. These findings indicate that there is a balance to maintain between microbial safety and quality preservation in specific heat sensitive RTE that can be achieved by careful consumer of those kinetic parameters when designing a process.

10. Sensitivity and Uncertainty Evaluation

Our parameter estimates were also highly plausible (plotted as images95 percent intervals), which was also indicated by bootstrapping of our uncertainty analysis. The sensitivity analysis showed the z-values and the shape parameter p of the Weibull were most likely to influence the predicted lethality, particularly in a shifting situation. A tenth change in z correlated with a portfolio change that is approximately the second-log prediction of a microbial survival. The quintessential measure of this observation in Figure 7 is that parametric sensitivity is glorified in the inner plates. Rather, successful and safe thermal processing can only be achieved by proper estimation of temperature-dependence parameters to validate the process.

Discussion

The current state of investigation provides quantitative and mechanistic knowledge of microbial inactivation in ready-to-eat (RTE), food, and minimum-processed food foods subject to mathematical modeling principles. The results, as a whole, expressed in eight tabularised results and the accompanying figures, show that the phenomenology of thermal death of concreteness food systems vary vastly with the first-order behaviour of the hypothetical, modern framework. These deviations are; food matrix make-up, physiology interruptions, and non-isothermal temperature discontinuities. Such results are also related to the recent tendencies in the predictive microbiology sphere that value non-linear and heterogeneous death kinetics support of simple models through flexibility (Martins et al., 2023; Xu and Zhang, 2022).

1. Evaluation of Modeling Approaches

The contrasting indications in the log-linear model, the Weibull model and biphasic model when discussing a course of study means that it is not feasible to skip non-linear expressions when verifying the process under investigation. The Weibull model also fits shoulders and tails of the observed survival curves with a resulting root mean square error that dropped greater than 30 percent further than the log-linear fits. This confirms by thermal-quirky heat resistance thermal-quirky distribution-parameter thermal validates kinetics Is followed by thermal resistance of microbials thermalicle thermal-quirky do follow a single rate constant. In addition, the use of biphasic models revealed that they were convenient to make simulations of languages of the sporing population that incorporated the subpopulation stubborn, as it did not come out of the population despite the extended treatment period (Nakauma et al., 2021). The degree of residual survivability of the cultures in the cheese based-matrices would support the fact that surrogates of Clostridium botulinum requires less holding-time as an issue was already known before (Silveira et al., 2020).

2. Influence of Food Matrix and Composition

The data, both experimental and behavioral, was modeled to show the protective properties of these factors; fats, starches and low water activity. Since Table 1 and Figure 1 reveal that, on average, there was a greater survival of microbes in high-fat systems, it can be inferred that matrix composition also moderates the success of heat transfer and mass movement, as evidenced by the between-effects of these variables. The recent researches by Sánchez et al. (2023), Bahuguna et al. (2022), and others also showed that the lipids could block thermal diffusions and create micro-zones of thermal protection, which prolongs D-values. The high positive correlation of the water activity of the matrix with the persistence of survivors observed corresponds to the findings of Heo et al., who found that survivor persistence is triggered by the low water activity that stabilised the microbial membrane against heat denaturation (2021). It follows that homogenous matrix-specific thermal and diffusional parameters must be taken into account to reflect a realistic model of the RTE foods in the light of the non homogenous microstructure of the actual products.

3. Thermodynamic and Kinetic Implications

A replotting of Arrhenius plots versus dependence on temperature, showed 358 kJ mol with minus or 459 kJ mol with minus respectively was an activation energy with the same origins as of solutions in the case of the crown activation in the literature. The high tolerance of the ln k vs. 1/T plots (Figure 3) suggests that the thermally activated processes promote inactivation in the microbes within the operated range. Rates of death exponentially accelerated with small temperature differences - a law that allows the processes to be maximized. Nevertheless, too steep inclines also expose oversensitivity of vile weather and, here, a minor discrepancy in homogeneity of the coloring can have an unbalanced deadly impact (Mañas et al., 2023). Therefore we require the need to tame the temperature and to filter-tune the model correction, finding the favourable safety we want without its implausible processing.

4. Spore Resistance and Multi-Phase Behavior

In the discovery of the physiologically differentiated subpopulation was the spores in which death occurred in a manner which may be termed biphase. As will be observed, Table 6 and Figures 5D6, a relatively small fraction of the spores, though still living, still required considerably more energy to be killed than with lower temperatures. Many changes of core hydration, mineralization and cross-linkage of proteins in spores of types differing in kind can explain this kind of behavior (Borges et al., 2022). It is these recalcitrant portions of k 2 2 that contain the long tails and require time to retain them compared with the normal pasteurizing conditions. Similarly, Phan-Thanh (2021) and Cao et al. (2022) could discover their findings on Bacillus cereus and Clostridium sporogenes in emulsion system respectively. These results serve to strengthen the infirmity of the single-phase models and the value of the biphasic or distributed-resistance models in determining the respondences of spores as microorganisms to the barium respondent in the industrial applications of RTE.

5. Model Validation under Non-Isothermal Conditions

Empirical experiments began to show that the Weibull model gave the most predictive values with a variety of temperature profiles. The outcome in the range below 1.0 and above 1.0 CHAT bias, whereas the result in Figure 7 is 1.1; both with high predictive reliability. This is related to the recently published results of Foster et al. (2021) and Bhattacharya et al. (2022), who have shown that the traditional D-z extrapolations over-globalize lethality patterns in the context of non-isothermic heating because it does not relate to transient postponement effects. In this calculation where deaths occur in an instant feel, much closer approximations of the actual world industrial heating profiles are obtained in the computer simulation. The advantage of the integrative model of Weibull-Arrhenius to represent food safety on a practical basis is that this data enables the same results to be predicted using the number of survivors as the level does.

6. Sensitivity and Uncertainty of Model Parameters

Sensitivity analysis revealed z-value and shape parameter p of the Weibull were the significant variables in the lethality prediction. The z-change that values calculated under nearly 0.7 logs of the anticipated decrease was the z-scaling z by 10 percent, which in consequence is explicable by the very fact that, as demonstrated in Dhowlaghar et al. (2023), the total model variation can principally be driven by the uncertainty in z-values. The result of the bootstrap confidence interval was low in this study that quantify identifiability of parameters and consistency of experiment were good. The findings of Brown and Chen (2024) were similar regarding Monte Carlo simulation of kinetics of Listeria in milk systems. The constructed models are highly robust and can be extended to a comparable matrix when applied to the experiment results with this minor error in 8 and this error in the activation energy.

7. Process Optimization and Industrial Relevance

Limitations of values are recalculated to produce moderate temperature increments pushing to non- quadratic reductions of holding-time requirement (Table 8, Figure 8). In one of these, an increase in processing temperature (68C to 70C) resulted in a reduction in the recommended hold time that was almost one-fifth that of the fundamental hold time, and that energy saved was not associated with a safety cost. They agree with predictive optimization framework suggested by Xu et al. (2023) and establish that pasteurization/sterilization time schedule combination is tuned with incorporation of kinetic modeling and thermal property evidence. The model-based design can be applied to the industry through a collection of vacant digital twins that are constantly creating parallel lethality in dynamic processes (Harvey et al., 2024). And, the quality of retention of products of the RTE with both model of microbial death and model of nutrient and texture loss (i.e., any of the two) is multi-objective and decided with a positive influence.

8. Broader Implications for Predictive Microbiology

The results aid the general transformation of predictive microbiology into becoming spatially data driven with modeling of a mechanistic-ordered style. The excellence of the non-linear and biphasic models in reports helps to justify the current attempts to merge the highest developed modeling tools in the international risk assessment guidelines (ICMSF, 2023). In addition, the results confirm that the under- and over-processing may be reduced to the lowest possible rate, by implementing the information basing on the experiment and statistics and calculations, to reduce the risk of going against the safety norms, sacrificing the quality of the product after which the safety standards are not going to beIntroduction to a new collection of moleshaving made (Patel et al., 2021). Mechanistic kinetics in this example is shown to be potentially useful in introducing corrections in processing conditions relative to real-time sensor inputs with extensions to machine-learning predictors (Ghosh et al., 2023).

9. Limitations and Future Research.

Their modeling, regardless of its tendency to reflect the underlying kinetic processes, requires additional investigation with added effects of synergy between pH and pressure as well as natural antimicrobials. The thermal processes in multi component systems most frequently influence the impact on permeability of the cell membrane and enzyme stability, influences the mortality of microorganisms and the quality of food (Singh et al., 2024). Moreover, a disadvantage to forecasting is microscopic dispersion of heat, especially in particulate foods or layered foods. Further models may be more accurate, incorporating computational fluid dynamics and thermal micro-scall imaging, to some extent. Last but not least, a probabilistic uncertainty study using massive industry data will mean adding the confidence of risk-based process validation systems.

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