



MODELLING THE GROWTH OF *SALMONELLA* IN FRUIT PUDDING AT DIFFERENT STORAGE TEMPERATURES

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ABSTRACT

Salmonella is one of the leading causative pathogens of foodborne diseases worldwide. While commonly associated with meat and poultry, egg-based desserts such as fruit pudding have also been linked to *Salmonella* outbreaks in Malaysia. This study investigated the growth behaviour of *Salmonella* in fruit pudding stored at 4°C and 25°C. The objectives were to evaluate suitable growth models, compare microbial counts on Bismuth Sulphite Agar (BSA) and Xylose Lysine Deoxycholate (XLD) agar, and assess physicochemical characteristics, including water activity, pH, and total soluble solids. Fruit pudding samples were inoculated with a *Salmonella* strain and stored under the specified temperatures. Microbial growth was monitored through periodic sampling, and enumeration on BSA and XLD agars, while physicochemical parameters were measured using standard methods. Growth data were fitted to selected primary models for comparison. The Huang model provided the best fit at 25°C, whereas a No Lag Phase model behaviour was observed at 4°C. Comparable *Salmonella* counts were obtained on both BSA and XLD agar, indicating similar detection performance. The physicochemical characteristics of the fruit pudding were found to support *Salmonella* growth under favourable conditions. These findings contribute to the development of predictive models of risk estimation and improved food safety management of dessert products. Future studies should explore *Salmonella* behaviour in a wider range of desserts, incorporate advanced molecular techniques, and evaluate natural preservatives and innovative packaging technologies to mitigate contamination risks.

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Introduction

Salmonella is a significant foodborne pathogen associated with egg products, which represent an economical source of dietary protein. Fecal matter from the poultry processing environment is a major route for *Salmonella* contamination on shelled eggs. The consumption of raw or undercooked eggs poses a substantial risk as

evidenced by a study by Wong *et al.* (2021) who reported the high probability of acquiring *Salmonella* Enteritidis through the consumption of half-boiled and minimally cooked eggs in Malaysia, via quantitative microbiological risk assessment approach.

Pudding is a widely consumed dessert globally, and its structure may act as a possible niche for *Salmonella* development. Moisture-sensitive foods like milk and eggs provide good conditions for microbial growth (Finn *et al.*, 2013). Starches and sugars are also energy sources, and such physicochemical characteristics as water activity (0.85–0.98) and pH (5.5–6.5) allow pathogen survival (Kristanti & Herminati, 2021). In Australia, *Salmonella* Typhimurium have been traced in 9 out of 12 stool samples from those who had consumed tiramisu that was made using raw eggs (Reynolds *et al.*, 2010). In Malaysia, home-prepared egg pudding has caused suspected Salmonellosis cases in 2020 and 2024, due to the use of raw egg white as a major ingredient in the pudding.

Storage temperature significantly influences egg safety and quality. Ambient storage temperature, which is usually between 20°C and 25°C, may encourage rapid *Salmonella* growth especially between 4 and 60°C which is widely considered to be “the danger zone” (Khan *et al.*, 2021). Maintaining appropriate temperature control is a major challenge for tropical countries like Malaysia as refrigeration during the supply chain of eggs can be costly. Furthermore, improper handling, cooking and storage of foods among Malaysians have been evidenced by multiple *Salmonella* outbreaks as reported in the local news in these recent years (BERNAMA, 2020; Abdullah, 2024; The Star, 2024; The Sun, 2024).

Predictive microbiology uses mathematical models to be able to predict the behaviour of microbes under given conditions of temperature, pH, and water activity. They are used in Hazard Analysis and Critical Control Point (HACCP) models, which help in hazard analysis and risk assessment to support safe storing and food safety measures (Taiwo *et al.*, 2024). Nevertheless, very little is known about the growth kinetics of *Salmonella* in fruit pudding when stored under various conditions.

Due to recurrent *Salmonella* outbreaks linked to eggs in Malaysia and the recognised role of time–temperature abuse in outbreak

settings, the purpose of the study was to compare and contrast the growth of *Salmonella* in fruit pudding stored at 4°C and 25°C, to determine variations in *Salmonella* count on Bismuth Sulphite Agar (BSA) and Xylose Lysine Deoxycholate (XLD) agar at various sampling points, and to compare physicochemical properties of fruit pudding including water activity, pH, and total soluble solids.

Materials and Methods

Sample Preparation

Fruit pudding was made according to a modified version of Hussein *et al.* (2023), where the use of pasteurized milk (500 mL), granulated sugar (100 g), cornstarch (40 g), vanilla extract (1 teaspoon), fresh-cut mixed fruits (200 g), fresh lemon juice (1 tablespoon), and one Grade B egg were used. All ingredients were purchased at Mydin Mall Gong Badak, Terengganu. The mixture was heated at 80°C for approximately four minutes until thickened and then cooled until it reached room temperature, 25°C, and then refrigerated for at least 1 hour before use. Control samples were prepared using uninoculated samples.

Physicochemical Analysis

Total Soluble Solids (TSS) were determined using a handheld refractometer (Pocket PAL-1, ATAGO Co., Tokyo, Japan) of the fruit pudding. A 10 g homogenized sample was calibrated using distilled water, and 1–2 drops were put on the prism and directly read the sample in the degree of °Brix. The measurements were done in triplicate to be accurate. Fruit pudding water activity (a_w) was determined with a water activity meter of calibrated standards (AquaLab 4TE, Meter Group, USA). A 10 g sample was placed in the sample cup, equilibrated in the chamber until a stable reading was obtained, and recorded. Measurements were performed in triplicate and averaged. The pH of fruit pudding was determined by homogenizing 10 g of sample with 90 mL distilled water. After calibration of the pH meter (Mettler Toledo FE20, Zurich,

Switzerland) with standard buffers (pH 4.0 and 7.0), the electrode was immersed in the mixture until a stable reading was obtained. Measurements were performed in triplicate and averaged.

Inoculum Preparation

Frozen *Salmonella* stock was obtained from the Food Microbiology Laboratory, Universiti Malaysia Terengganu, and cultured in Tryptic Soy Broth (TSB) at 37°C for 24 hours. An aliquot was transferred to Tryptic Soy Agar (TSA) and incubated at 37°C for 24 hours. A single colony was inoculated into 5 mL TSB and incubated for 12 hours. Cells were harvested by centrifugation (2500 rpm), washed twice with one-quarter strength Ringer's solution, and adjusted to approximately 3–4 log₁₀ CFU/mL. All of the culture media was obtained from Oxoid (UK).

Inoculation and Incubation

Uninoculated fruit pudding samples served as controls to determine background microbiology (Feng *et al.*, 2024). Ten grams of sample were homogenized with 90 mL Buffered Peptone Water (BPW), serially diluted and 0.1 mL aliquots were plated on selective media. Media were incubated at 37°C for 24 hours for *Salmonella* enumeration as log₁₀ CFU/g (Feng *et al.*, 2024). For inoculated samples, 10 g of fruit pudding was mixed with 100 µL of *Salmonella* suspension (3–4 log₁₀ CFU/mL), transferred into sterile stomacher bags, and stored at 4°C and 25°C. Bacterial populations were monitored at designated time points until reaching the stationary phase, with all samples analysed in duplicate.

Determination of Salmonella

At each sampling point, 10 g of fruit pudding was homogenized with 90 mL Buffered Peptone Water (BPW), serially diluted, 0.1 mL aliquots were plated onto Bismuth Sulphite Agar (BSA) and Xylose Lysine Deoxycholate (XLD) agar and incubated at 37°C for 24 hours. On XLD, *Salmonella* appeared as colonies with black centers due to hydrogen sulphide production, while on BSA they appeared black or dark brown due to bismuth sulphide reduction. Results were expressed as log₁₀ CFU/g using the formula: Log (number of colonies × dilution factor) / volume plated (mL). The use of both media allowed for broader recovery of *Salmonella* species, as nutrient compositions may differentially support their growth (Park *et al.*, 2012).

Growth Model Development

Growth data at 25°C were fitted to the Baranyi, Huang, Modified Gompertz, and Buchanan three-phase linear models, while data at 4°C were fitted to the No Lag Phase, Reduced Huang, Reduced Baranyi, and Buchanan two-phase linear models. Model fitting and statistical evaluation were conducted using the Integrated Pathogen Modelling Program (IPMP 2013, United States Department of Agriculture USDA-ARS), a freely available software at <https://www.ars.usda.gov/>. Figure 1 presents the graphical user interface (GUI) of IPMP 2013.

The Baranyi model describes microbial growth by incorporating both the lag and exponential phases, with parameters representing the initial population size (Y_0), maximum population (Y_{max}), specific growth rate (μ_{max}), and physiological state of the microorganism (h_0) (Baranyi & Roberts, 1995). The mathematical expression for the Baranyi model is as follows:

The Huang model is commonly applied to

$$Y(t) = Y_0 + \mu A(t) - \ln \ln \left\{ 1 + \frac{\exp \exp [\mu A(t)] - 1}{\exp \exp (Y_{max} - Y_0)} \right\}$$

$$A(t) = t + \frac{1}{\mu_{max}} \ln [\exp \exp (-\mu_{max} t) + \exp \exp (-h_0) - \exp (-\mu_{max} t - h_0)]$$

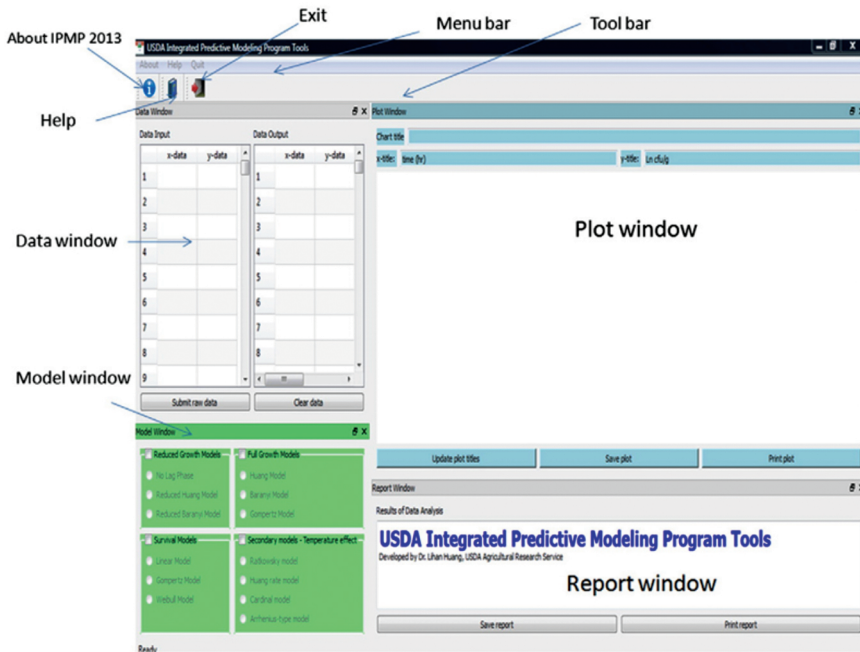


Figure 1: Graphical User Interface (GUI) for IPMP 2013

Source: Huang (2014)

describe bacterial growth curves that exhibit distinct lag, exponential, and stationary phases. It estimates such parameters as initial (Y_0) and maximum population (Y_{max}), specific growth rate (μ_{max}), lag phase length (λ), and transition

coefficient (α) that control the change between lag and exponential growth. The formula for the Huang model is as follows:

The sigmoidal bacterial growth curves are

$$Y(t) = Y_0 + Y_{max} - \ln \ln \{e^{Y_0} + [e^{Y_{max}} - e^{Y_0}]e^{-\mu B(t)}\}$$

$$B(t) = t + \frac{1}{a} \ln \frac{1 + e^{-\alpha(t-\lambda)}}{1 + e^{a\lambda}}$$

extensively explained by the Modified Gompertz model, which explains lag, exponential, and stationary growth phases. Its parameters are the initial (Y_0) and the maximum population size (Y_{max}), the particular rate of growth (μ_{max}), and the lag period (λ). It also has an inflection point,

which is where the rapid growth turns into the stationary phase, and thus the model is especially useful when fitting the microbial growth data of the experiment. The modified Gompertz model formula is usually expressed as:

The Three-phase Linear model describes

$$Y(t) = Y_0 + (Y_{max} - Y_0) \exp \exp - \left[\frac{\mu_{max} e}{Y_{max} - Y_0} (\lambda - t) + 1 \right]$$

bacterial growth in three distinct stages: Lag, exponential, and stationary. It takes the parameters of initial population (Y_0), the maximum population ($Y_{\max}$), and the growth rate (k), and $t_{\max}$ is the time at which the population will be at the maximum. This model is a good

way of demonstrating the stage between lag and exponential growth, and the stabilizing stage, which is the stationary one. The model is expressed as follows:

The No Lag Phase model describes

$$\begin{aligned} y &= y_0, \text{ if } t < \text{lag} \\ y &= y_0 + k(t - \text{lag}), \text{ if } \text{lag} \leq t < t_{\max} \\ y &= y_{\max}, \text{ if } t \geq t_{\max} \end{aligned}$$

bacterial growth curves that transition directly into exponential growth without a lag period. The most important parameters are the initial (Y_0) and the maximum ($Y_{\max}$) population, the population at a point in time t [$Y(t)$], and the particular growth rate ($\mu_{\max}$). In conditions

where there is minimal adaptation of microbes with rapid proliferation, this model is helpful in describing the growth of microorganisms. The equation for the model is expressed as:

The Reduced Huang model is a

$$Y(t) = Y_0 + Y_{\max} - \ln \ln [e^{Y_0} + (e^{Y_{\max}} - e^{Y_0})e^{-\mu_{\max}t}]$$

simplification of the Huang model, which is appropriate to growth curves that never enter a stationary point. It estimates such parameters including the initial population (Y_0), maximum population ($Y_{\max}$), specific growth rate ($\mu_{\max}$),

and lag phase duration (λ), making it useful for conditions where bacterial growth slows but does not stabilize at a stationary phase. The Reduced Huang model equation is:

The simplified form of the Baranyi model

$$Y(t) = Y_0 + \mu_{\max} \left[t + \frac{1}{4} \ln \ln \left(\frac{1 + e^{-4(t-\lambda)}}{1 + e^{4\lambda}} \right) \right]$$

is the Reduced Baranyi model, which is used when some of the growth parameters can be simplified. It includes the starting population (Y_0), top population ($Y_{\max}$), the particular growth rate ($\mu_{\max}$), and the physiological state parameter (h_0), providing an easy method to

model microbial growth under circumstances in which the behaviour of bacteria is less intricate (Baranyi & Roberts, 1995). The Reduced Baranyi model equation is:

The Two-phase Linear model explains the

$$Y(t) = Y_0 + \mu_{\max}t + \ln(e^{-\mu_{\max}t} + e^{-h_0} - e^{-\mu_{\max}t-h_0})$$

growth of microbes by the exponential phase that is preceded by a lag phase and does not reach a stationary phase. Parameters are initial population (Y_0), lag phase (λ), growth rate (k), and population maximum ($Y_{\max}$). The model has found application especially in modelling

growth curves in which bacteria will switch directly between adaptation and high growth processes (Buchanan *et al.*, 1997). The Two-phase Linear Growth model equation is given as:

Statistical Analysis

$$\begin{aligned} y &= y_0, \text{ if } t < \text{lag} \\ y &= y_0 + k(t - \text{lag}), \text{ if } t \geq \text{lag} \end{aligned}$$

Growth models of *Salmonella* in fruit pudding at both 4°C and 25°C were fitted and compared using the Integrated Pathogen Modelling Program (IPMP). Root Mean Square Error (RMSE) was used to assess model performance, with lower values being a better fit. Paired t-tests in Minitab were used to determine differences in the count of *Salmonella* on Bismuth Sulphite Agar (BSA) and Xylose Lysine Deoxycholate (XLD) agar using the significance level of $p < 0.05$.

Results and Discussions

Physicochemical Characteristics of Fruit Pudding

Table 1 shows the physicochemical characteristics of the fruit pudding. The mean pH was 6.28 ± 0.01 , water activity (a_w) was 0.986 ± 0.002 , and the total soluble solids (TSS) were 33.7 ± 0.5 °Brix.

The physicochemical study has revealed that the fruit pudding is favourable to the growth of microorganisms because the pH was more than the minimum level of pathogen growth ($pH > 4.2$), which means that it does not possess any inherent anti-inhibitory components (Feng *et al.*, 2024). The pH of 6.28 is slightly acidic to near neutral, which is probably due to the use of slightly acidic fruits like apples and pears as ingredients (Feng *et al.*, 2024). This is a good value of the *Salmonella* growth since biofilm formation is maximized at neutral pH (Papakonstantinou & Efthimiou, 2020).

The value of water activity (0.986) is high, indicating the presence of a high amount of free water that favours the growth of microbes. The pudding is a conducive environment to the growth of *Salmonella* since it can be grown

at raw values of more than 0.90 (Eriksson de Rezende *et al.*, 2001).

The TSS of 33.7 °Brix means that the concentration of sugars and other dissolved solids is rather high. The high TSS can be linked to the high availability of nutrients, which can also promote the growth of bacteria under favourable temperature and pH conditions (Singh & Yemmireddy, 2021). All these physicochemical characteristics show the possible danger of *Salmonella* growth in fruit pudding, provided it is not stored and handled properly.

Background Salmonella in Fruit Pudding

No colonies were observed on either BSA or XLD agar in the uninoculated control samples. The absence of growth indicates that the fruit pudding samples were free from detectable microbial contamination at the start of the experiment, suggesting effective sanitation or inherently clean raw materials (Feng *et al.*, 2024). This observation is necessary because it proves that the observed further *Salmonella* growth in the inoculated samples can be explained only by experimental inoculation and storage conditions, but not by the presence of contamination.

The control samples must have no microbiota background: This is a critical validation requirement when performing microbiological studies to ensure the credibility of the findings. Other researchers have used similar methods, including (Hussein *et al.*, 2023), where uninoculated rice pudding was used to test that the growth of microorganisms in experimental samples was due to intentional inoculation and not contamination.

Table 1: Physicochemical characteristics of fruit pudding

Physicochemical Parameters	Mean ± S.D.
pH	6.28 ± 0.01
Water Activity, a_w	0.986 ± 0.002
Total Soluble Solids (°Brix)	33.7 ± 0.5

Note: Standard Deviation (SD) (n =3)

Modelling Growth Kinetics of Salmonella in Fruit Pudding at 25°C

The predictive model was established based on the *Salmonella* enumeration data collected on Xylose Lysine Deoxycholate (XLD) agar reported to have high sensitivity (92.7%) in the detection of *Salmonella* in food samples (Park et al., 2012). *Salmonella* in fruit pudding was assessed by four models, namely Huang, Baranyi, Modified Gompertz, and Buchanan Three-phase Linear at 25°C. The application of these growth models was used to compare and explain

the growth of the bacteria under these given conditions. The growth parameters estimated for each model are summarised in Table 2, and the actual growth curves, depicting the observed and the predicted growth, are given in Figures 2 to 5. These models provide information on the lag stage, maximum specific growth rate, and reach density, which is important in this case to determine the behaviour of *Salmonella* in the fruit pudding at ambient temperature.

Table 2: Growth parameters of the Huang model, Baranyi model, Modified Gompertz model, and Buchanan Three-phase Linear model to predict the growth of *Salmonella* at 25°C

Parameters	Value	Std-Error	t-Value	p-Value	L95CI	U95CI
Huang Model						
Y _o	8.244	1.096	7.523	1.671E-03	5.201	11.287
Lag	0.244	4.522	0.054	9.596E-01	-12.312	12.800
Y _{max}	19.583	7.188	2.724	5.275E-02	-0.374	39.540
μ _{max}	0.424	0.073	5.786	4.432E-03	0.220	0.627
Baranyi Model						
Y _o	9.701	0.695	13.961	1.527E-04	7.772	11.630
h _o	17.643	16.455	1.072	3.440E-01	-28.043	63.329
Y _{max}	17.908	1.256	14.263	1.404E-04	14.422	21.394
μ _{max}	1.336	0.968	1.379	2.398E-01	-1.353	4.024
Modified Gompertz Model						
Y _o	9.703	0.702	13.823	1.588E-04	7.754	11.652
Lag	13.829	2.342	5.906	4.114E-03	7.328	20.330
Y _{max}	18.059	1.572	11.490	3.275E-04	13.695	22.422
μ _{max}	1.710	1.441	1.186	3.012E-01	-2.292	5.711
Buchanan Three-phase Linear Model						
Y _o	9.166	0.767	11.951	2.808E-04	7.037	11.295
k	0.496	0.146	3.404	2.719E-02	0.091	0.901
Lag	4.583	3.804	1.205	2.947E-01	-5.978	15.144
Y _{max}	17.825	1.328	13.419	1.784E-04	14.137	21.513

Note: Y_o = initial microbial counts; Lag = lag time (h); Y_{max} = maximum microbial counts; μ_{max} = specific growth rate (time⁻¹); h_o = physiological state of the microorganism; k = specific growth rate

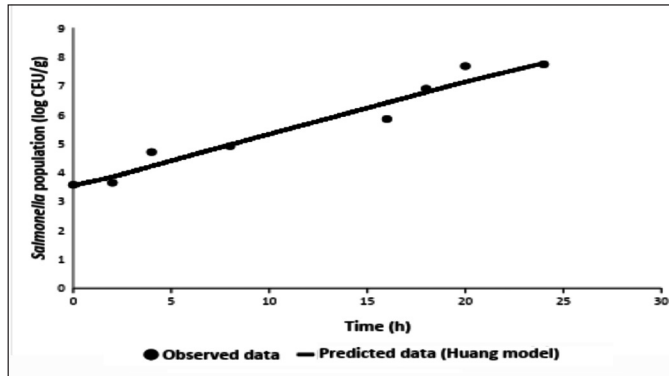


Figure 2: *Salmonella* growth curve at 25°C observed and predicted data for Huang model

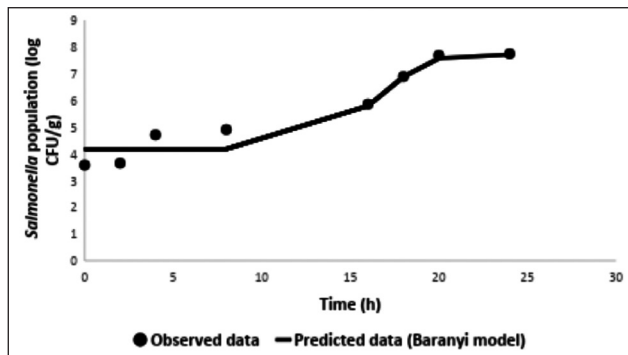


Figure 3: *Salmonella* growth curve at 25°C observed and predicted data for Baranyi model

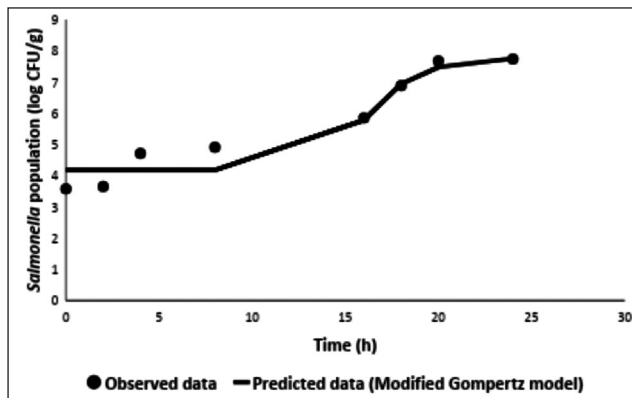


Figure 4: *Salmonella* growth curve at 25°C observed and predicted data for Modified Gompertz model

The initial microbial counts of *Salmonella* (Y_0) predicted by the four models ranged from 8.244 to 9.703. Y_0 represents the microbial population size at the beginning of the growth experiment and serves as a critical baseline for

assessing population dynamics over time (Li *et al.*, 2020).

The Huang model gave the smallest initial population ($Y_0 = 8.244$), indicating the lower initial concentration of *Salmonella* and the

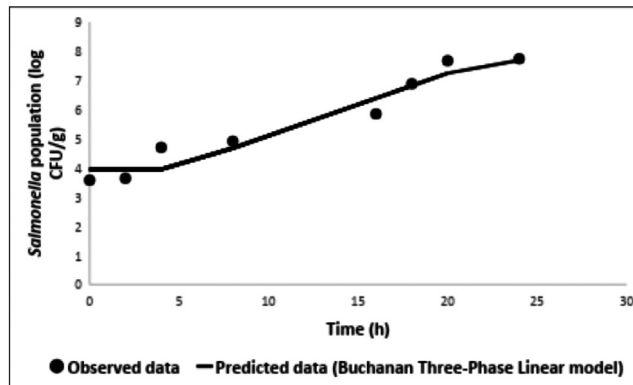


Figure 5: *Salmonella* growth curve at 25°C observed and predicted data for Buchanan Three-phase Linear model

shortest lag phase until the exponential growth. This was in agreement with the predicted lag time in the model of 0.244 h. This behaviour is consistent with the fact that the Huang model can be used in systems with rapid adaptation of bacteria and quick passage to exponential growth (Anandita *et al.*, 2024).

In comparison, the Baranyi ($Y_0 = 9.701$) and the Modified Gompertz ($Y_0 = 9.703$) models forecasted more initial counts, as the two models are more detailed in terms of lag-to-growth transitions. This is explained by the Baranyi model, which uses a mechanistic explanation of microbial behaviour by considering the mechanisms of bacterial adaptation during the lag phase (Grijnspeerdt & Vanrolleghem, 1999). The Buchanan Three-phase Linear model forecasted a mid-range Y_0 (9.166) and indicates a simple empirical structure and use of a simple phase segmentation (Grijnspeerdt & Vanrolleghem, 1999).

Maximum population density ($Y_{\max}$) of *Salmonella* was quite similar in the models, which ranged between 17.825 and 19.583 log CFU/g. It was the Huang model that had the highest value ($Y_{\max} = 19.583$), which implied the existence of better conditions leading to the stability of the population during the stationary stage (Navarro Llorens *et al.*, 2010). The Buchanan Three-phase Linear model projected the minimum ($Y_{\max} = 17.825$), which takes into consideration environmental stress and

nutrient constraints that could limit the growth. The Modified Gompertz ($Y_{\max} = 18.059$) and Baranyi Model ($Y_{\max} = 17.908$) models yielded intermediate predictions, by being realistic and less complex curve-fitting models (Gil *et al.*, 2006).

The specific growth rate ($\mu_{\max}$) differed more significantly among the models, with values of 0.424 to 1.710 h⁻¹. According to the Huang ($\mu_{\max} = 0.424$) and Buchanan Three-phase Linear ($\mu_{\max} = 0.496$) models, the growth was moderate with steady but not stronger doubling exponential growth rates. However, the Baranyi ($\mu_{\max} = 1.336$) and the Modified Gompertz ($\mu_{\max} = 1.710$) models showed rapid growth, which implied highly favourable conditions with a population doubling more than once an hour in modelled conditions. These models have higher $\mu_{\max}$ values, which indicate the sensitivity of these models to environmental conditions and the ability to describe rapid microbial growth (Khasa & Mohanty, 2021).

All these findings support the various assumptions and strong points of each predictive model. Although the Huang model worked well in high-adaptation speed with a large $Y_{\max}$, the Baranyi and Gompertz model showed more elaborate growth kinetics, especially in terms of lag and exponential-phase behaviour. The less complicated Buchanan Three-phase Linear model, however, provided practical insights with the conservative estimates of growth behaviour.

Modelling Growth Kinetics of Salmonella in Fruit Pudding at 4°C

In order to have a clearer understanding of the predictive behaviour of *Salmonella* at 4°C, various predictive models were tested in this study. These models were the Reduced Huang, No Lag Phase, Reduced Baranyi, and Buchanan Two-phase Linear models. These models are also known to forecast the growth of microbes in a given environmental condition, especially in food safety and food refrigeration. They were chosen according to their appropriateness to low-temperature modelling, and as such they were suitable for assessing the dynamics of *Salmonella* growth at 4°C. The analysis offers enough information on the predictive validity and performance of these models in ensuring the safety of food stored in cold conditions. Table 3 indicates the growth parameters of four models used to examine the growth of *Salmonella* at 4°C (Reduced Huang, No Lag Phase, Reduced

Baranyi, and Buchanan Two-phase Linear). In Figures 6 to 9, the predicted and observed *Salmonella* growth curve at 4°C is presented in Reduced Huang, No Lag Phase, Reduced Baranyi, and Buchanan Two-phase Linear models.

The Reduced Huang model predicted an initial *Salmonella* population (Y_o) of 8.107, with a short lag phase of 0.124 h. This very brief lag time suggests rapid adaptation of *Salmonella* to the pudding environment, although the model predicted a low maximum specific growth rate ($\mu_{max} = 0.048 \text{ h}^{-1}$). The reduced growth rate reflects the inhibitory effect of refrigeration on metabolic activity, as cold conditions slow enzymatic reactions essential for bacterial proliferation. Oscar (2014) similarly reported that *Salmonella* growth is supported at higher temperatures (12°C–16°C), whereas at lower

Table 3: Growth parameters of the Reduced Huang model, No Lag Phase model, Reduced Baranyi model, and Buchanan Two-phase Linear model to predict the growth of *Salmonella* at 4°C

Parameters	Value	Std-Error	t-Value	p-Value	L95CI	U95CI
Reduced Huang Model						
Y_o	8.107	0.773	10.483	4.681E-04	5.959	10.254
Lag	0.124	32.315	0.004	9.971E-01	-89.596	89.844
μ_{max}	0.048	0.008	6.073	3.715E-03	0.070	0.070
No Lag Phase Model						
Y_o	7.769	0.365	21.302	2.872E-05	6.756	8.781
Y_{max}	13.338	0.580	22.989	2.121E-05	11.727	14.949
μ_{max}	0.065	0.011	5.780	4.450E-03	0.034	0.096
Reduced Baranyi Model						
Y_o	7.951	0.623	12.754	2.178E-04	6.220	9.682
h_o	-0.550	1.428	-0.385	7.196E-01	-4.515	-4.515
μ_{max}	0.043	0.013	3.405	2.714E-02	0.008	3.414
Buchanan Two-Phase Linear Model						
Y_o	8.107	0.773	10.483	4.681E-04	5.959	10.254
k	0.048	0.008	6.073	3.715E-03	0.026	0.070
Lag	0.243	20.078	0.012	9.909E-01	-55.503	55.989

Note: Y_o = initial microbial counts; Lag = lag time (h); Y_{max} = maximum microbial counts; μ_{max} = specific growth rate (time⁻¹); h_o = physiological state of the microorganism k = specific growth rate

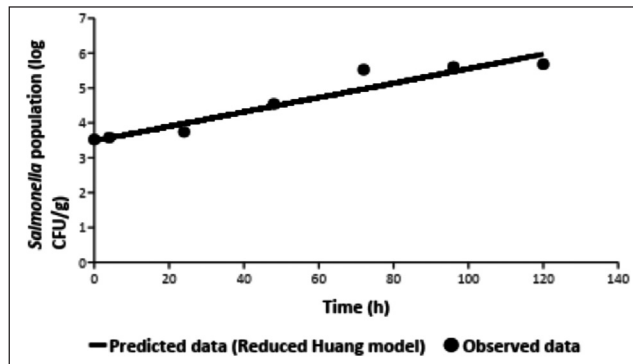


Figure 6: *Salmonella* growth curve at 4°C observed and predicted data for Reduced Huang model

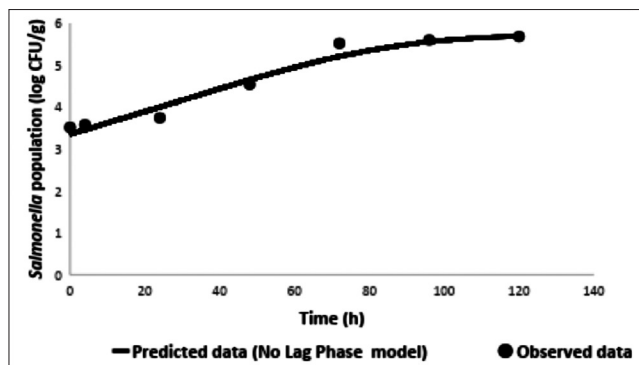


Figure 7: *Salmonella* growth curve at 4°C observed and predicted data for No Lag Phase model

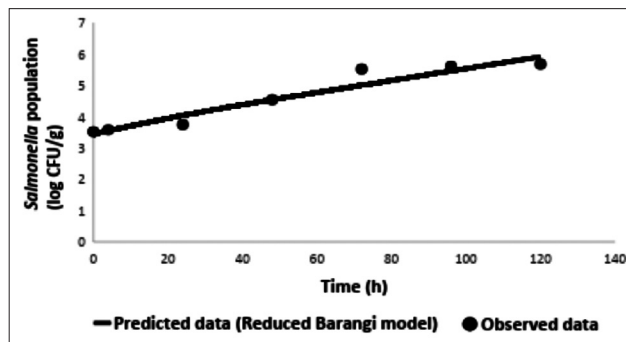


Figure 8: *Salmonella* growth curve at 4°C observed and predicted data for Reduced Baranyi model

temperatures (-8°C to 10°C), bacterial populations remain stable or decline slightly in chicken meat.

The No Lag Phase model estimated a slightly lower initial population ($Y_0 = 7.769$) compared to other models and predicted a maximum population ($Y_{\max}$) of 13.338. This

value is the plateau of the stationary phase when the growth is stopped by the lack of nutrients or the presence of waste. The $\mu_{\max}$ in prediction (0.065 h^{-1}) was larger than that of the Reduced Huang (0.048 h^{-1}) and Buchanan Two-phase Linear ($k = 0.048\text{ h}^{-1}$) models, and it is just that *Salmonella* cells start dividing without a lag period. The lack of a lag phase, therefore,

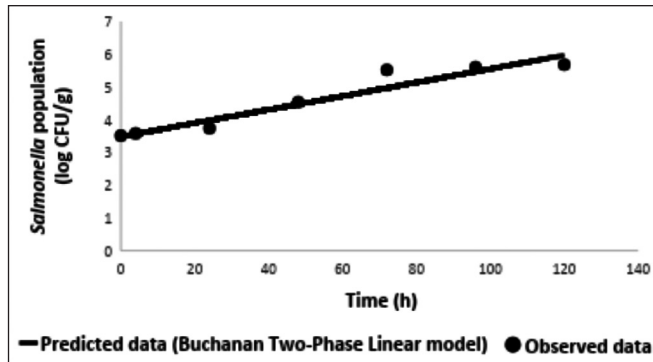


Figure 9: *Salmonella* growth curve at 4°C observed and predicted data for Buchanan Two-phase Linear model

increases effective growth, which mimics the conditions when the bacteria have adapted well to the environment.

The predicted initial population (Y_0) by the Reduced Baranyi model, 7.951, was in agreement with the other models. Notably, the model generated a negative value of the adaptation parameter ($h_0 = -0.550$), suggesting limited or negligible bacterial adaptation at 4 °C. The forecasted 0.043 h^{-1} is a very slow growth, and this corresponds with the low metabolic activity of refrigeration. These findings are consistent with other studies that found that the lowest growth temperature of *Salmonella* was 8°C–10°C in mung bean sprouts and 10°C in shredded cabbage salad (Son *et al.*, 2024), which confirms that the growth at 4°C is highly inhibited.

In the Buchanan Two-phase Linear model, the initial population was predicted to be 8.107, with a lag time of 0.243 h because the growth was broken down into lag and exponential phases. The modelled rate of growth ($k = 0.048 \text{ h}^{-1}$) was in agreement with the Reduced Huang model, which once again shows the reduction in growth rate caused by refrigeration on *Salmonella* multiplication. The model was simple, however, and it was able to explain growth under low-temperature conditions with the focus on the minimal metabolic activity of *Salmonella* in refrigerated conditions.

Together, the four models ensure the presence of heavily limited growth of *Salmonella* in fruit pudding at 4°C with extremely low values of $\mu_{\max}$ and minimal growth in population. This implies that refrigeration does not kill *Salmonella*, but it slows down its growth to a great extent, minimising, though not removing, food safety hazards.

Evaluation of Predictive Models for *Salmonella* Growth in Fruit Pudding at 25°C and 4°C

The predictive models were tested at 25°C and 4°C to determine the accuracy and reliability of the predictive models at both temperatures in simulating *Salmonella* growth at various temperatures of storage. To measure model performance, statistical measures such as the Sum of Squared Errors (SSE), Mean Squared Error (MSE), Root Mean Squared Error (RMSE), and residual standard deviation were applied. These indices provide an overall measure of the agreement between model predictions and observed data, and the smaller the index, the greater the model fit.

The results of Huang, Baranyi, Modified Gompertz, and Buchanan Three-phase Linear model are presented in Table 4, and the Reduced Huang, No Lag Phase, Reduced Baranyi, and Buchanan Two-phase Linear models are presented in Table 5 at the two temperatures of 25°C and 4°C, respectively.

Table 4: Evaluation of various models to predict the growth of *Salmonella* at 25°C

	Model			
	Huang	Baranyi	Modified Gompertz	Buchanan Three-phase Linear
SSE	4.804	7.721	7.884	7.058
MSE	1.201	1.930	1.971	1.765
RMSE	1.096	1.389	1.404	1.328
Residual stdev	0.775	0.982	0.993	0.939

Table 5: Evaluation of various models to predict the growth of *Salmonella* at 4°C

	Model			
	Reduced Huang	No Lag Phase	Reduced Baranyi	Buchanan Two-phase Linear
SSE	2.392	1.168	2.288	2.392
MSE	0.598	0.292	0.572	0.598
RMSE	0.773	0.540	0.756	0.773
Residual stdev	0.585	0.409	0.572	0.585

Note: SSE = Sum of squared errors; MSE = Mean of SSE; RMSE = Square root of MSE; Residual stdev = Standard deviation of errors

Sharma *et al.* (2021) indicate that the model that gives the lowest value of RMSE is the one with the best predictive performance. In the same vein, (Hodson, 2022) noted that values of RMSE will be low, which signifies that the predictions are more accurately approximated to the observed data, hence demonstrating greater model accuracy.

The Huang model produced the lowest RMSE value (1.096) at 25°C, which is the best fit of the four models. This implies that the Huang model is most predictive in forecasting the growth of *Salmonella* in fruit pudding during ambient storage. The No Lag Phase model yielded the lowest RMSE (0.540) at 4°C, which is a better predictive model than all the other models that were tried.

The lowest values of RMSE are 1.096 (25°C) and 0.540 (4°C), though the lowest of the respective models can be said to be relatively high. This may be explained by the biological variation in the reactions of microbes. Temperature changes, pH variations, and nutrient availability are other factors that have strong effects on the growth of micro-organisms,

and they normally bring about inconsistencies leading to the reduction of the precision of the model. Also, to dynamically respond to environmental stresses, microorganisms change metabolic activity, posing an even more difficult challenge to predicting them (Sharma *et al.*, 2021).

Differences in Salmonella Populations on XLD and BSA at 25°C

The population growth of *Salmonella* at 25°C was measured after 24 hrs in Xylose Lysine Deoxycholate (XLD) and Bismuth Sulphite Agar (BSA) to compare the count in both media. Sampling was done at 0, 2, 4, 8,16, 18, 20, and 24 hours to determine the changes in bacterial growth over time. A summary of the results is presented in Table 6.

To establish whether there were significant differences between the *Salmonella* populations enumerated on XLD and BSA at various sampling times (0–24h), paired *t*-test analysis was conducted. The results showed that there are no significant differences ($p > 0.05$) at 0 h ($p = 0.407$), 2 h ($p = 1.000$), 4 h ($p = 0.303$),

Table 6: *Salmonella* population, log CFU/g on XLD and BSA agar at 25°C across different sampling times (0, 2, 4, 8, 16, 18, 20 and 24 hours)

Time (Hour)	XLD agar (log CFU/g) Mean $\pm$ SD	BSA (log CFU/g) Mean $\pm$ SD
0	3.51 $\pm$ 0.099 ^a	3.71 $\pm$ 0.106 ^a
2	3.65 $\pm$ 0.021 ^a	3.69 $\pm$ 0.021 ^a
4	4.70 $\pm$ 0.106 ^a	4.53 $\pm$ 0.014 ^a
8	4.91 $\pm$ 0.057 ^a	4.64 $\pm$ 0.028 ^b
16	5.84 $\pm$ 0.071 ^a	5.89 $\pm$ 0.014 ^a
18	6.89 $\pm$ 0.042 ^b	7.51 $\pm$ 0.028 ^a
20	7.67 $\pm$ 0.071 ^a	7.60 $\pm$ 0.042 ^a
24	7.74 $\pm$ 0.064 ^a	7.78 $\pm$ 0.064 ^a

16 h ($p = 0.430$), 20 h ($p = 0.542$), and 24 h ($p = 1.000$), which implies that both media offer a similar recovery of *Salmonella* at these times. The population was almost equal at these time points, especially at 2 h and 24 h, where p -values of 1.000 also meant that the two media had virtually no difference.

There were differences of significance at the 8 h ($p = 0.047$) and 18 h ($p = 0.046$) time points. XLD agar yielded slightly higher counts than BSA at 8 h, which could be explained by the media differences. XLD agar includes thiosulfate and ferric ammonium citrate, which helps to identify production of hydrogen sulphide that is typical of *Salmonella* (Richardson *et al.*, 2019). BSA, on the other hand, contains bismuth Sulphite and brilliant green, which prevent competing bacteria, although they would temporarily lower recovery efficiency (D'aoust, 1977). At 18h, BSA produced more counts compared to XLD, which may indicate variations in nutrient supply and the influence of selective agents as *Salmonella* moved towards the late exponential or early stationary phase.

In general, the results show that both XLD and BSA are suitable for enumerating *Salmonella* with slight variations only at certain stages of growth. The consistency observed across most sampling times indicates the credibility of both media, whereas the differences observed indicate the impact of selective media composition on bacterial recovery.

Differences in Salmonella Populations on XLD and BSA at 4°C

The increase in the population of *Salmonella* at 4°C was observed after 120 h to compare the recovery of the population with XLD and BSA agars. A sampling interval of 0, 4, 24, 48, 72, 96, and 120 h was used to sample a population when it was kept cold in storage. The efficiency of detection was compared in both selective media at refrigeration conditions. Table 7 demonstrates the population of *Salmonella* (log₁₀ CFU/g) on the XLD and BSA agars at each of the times.

The paired *t*-test analysis result indicated that there were no significant differences in *Salmonella* populations of XLD and BSA at any point of the sampling. The p -values were 0.278 (0 h), 0.228 (4 h), 1.000 (24 h), 0.553 (48 h), 0.795 (72 h), 1.000 (96 h), and 1.000 (120 h), all above the significance threshold of 0.05. These findings show that although there were slight differences in the mean values, the differences did not prove to be significant at any given time. All in all, XLD and BSA can equally be used to support *Salmonella* enumeration regardless of storage temperature, and most importantly indicates that *Salmonella* growth was not affected by recovery media. While the use of selective media is desirable to suppress background microflora growth, *Salmonella* growth rate could be overestimated or underestimated if composition of selective media influenced *Salmonella* growth.

Table 7: *Salmonella* population, log CFU/g on XLD and BSA agar at 4°C across different sampling times (0, 4, 24, 48, 72, 96 and 120 hours)

Time (Hour)	XLD Agar (log CFU/g) Mean ± SD	BSA (log CFU/g) Mean ± SD
0	3.51 ± 0.028 ^a	3.59 ± 0.021 ^a
4	3.58 ± 0.021 ^a	3.66 ± 0.021 ^a
24	3.74 ± 0.042 ^a	3.74 ± 0.028 ^a
48	4.55 ± 0.035 ^a	4.60 ± 0.057 ^a
72	5.51 ± 0.028 ^a	5.50 ± 0.014 ^a
96	5.60 ± 0.028 ^a	5.53 ± 0.028 ^a
120	5.68 ± 0.028 ^a	5.60 ± 0.028 ^a

Conclusions

Overall, the findings demonstrated that the type of selective medium did not significantly influence *Salmonella* enumeration in the fruit pudding matrix. The Huang model was identified as the most suitable model for predicting *Salmonella* growth at 25°C, whereas the No Lag Phase model provided the best fit at 4°C., based on the lowest RMSE values. This study emphasizes *Salmonella* growth in fruit pudding under a worst-case scenario (25°C) and common storage temperature at household settings (4°C), when contaminated or uncooked eggs were used for making the dessert. Nevertheless, there are several limitations for this study. First, this study only focused on primary models for two storage temperatures. Minimum, optimal and maximum growth temperature can be further determined using a secondary model if multiple temperatures were tested. Following that, model validation can be performed to further justify the accuracy of the model. Second, manually-cracked whole eggs were used in this study, which may not represent commercial production methods, due to biological and physical hazards. It is suggested to incorporate liquid egg products and further study the impact of liquid egg composition and source of liquid eggs to improve the understanding of *Salmonella* growth in local delicacies in Malaysia.

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Author Contributions Statement

ZYL conducted the research, drafted the manuscript, and prepared the revisions. MNL and NHA conceptualised the research idea and supervised the overall progress. MYFJ and ESM contributed to the research design and development of the framework. NH led the review process, revisions and approved the final manuscript for submission.

Conflict of Interest Statement

The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts.

Ethical Conduct

Ethical approval and informed consent were not required because the study did not involve human participants or animals.

Declaration of AI-usage

During the preparation of this work, the authors used AI-tools for grammatical improvements and clarity. In using the AI-tools, the authors reviewed and edited the content as needed and bear full responsibility for the content of this manuscript.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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