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Revisiting secondary model features for describing the shoulder and lag parameters of microbial inactivation and growth models

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ABSTRACT

The Baranyi and Geeraerd models are two of the most reliable models for the description of, respectively, microbial growth and inactivation. They are defined as a system of differential equations, whose algebraic solution can describe the microbial response during isothermal conditions, especially when combined with suitable secondary models. However, there are still large uncertainties regarding the best functions to use as secondary models for the lag phase duration (λ) and the shoulder length (S_l).

In this article, we revisit these models, focusing on the implications related to the assumption of an ideal substance whose dynamics define bacterial adaptation. We demonstrate that their link with the isothermal lag and shoulder leads to unique secondary models for the effect of temperature changes on λ and S_l. Namely, a log-linear relationship for S_l and a reverse cuadratic relationship for λ (considering a Ratkowsky model for μ). Furthermore, we observe a coupling between both secondary models (k and S_l for Geeraerd; λ and μ for Baranyi), reducing the number of unknown model parameters from four to three. Using data from the scientific literature, we illustrate the applicability of these results, being able to improve the robustness of parameter estimates.

The identification of these links are of great relevance for the field of predictive microbiology, as they resolve the uncertainty regarding the functional form of secondary models. Our results also provide a way to assess the validity of those dynamic hypotheses using data gathered under isothermal conditions, something that was hardly possible using data gathered under dynamic conditions. Although this study is limited to the effect of temperature, the general approach and methodology are also applicable to other type of secondary models, so this article can be a blueprint for future studies.

1. Introduction

Predictive microbiology can be considered a standard within the toolbox of the modern food microbiologist. This field develops mathematical models that are able to predict how microbial populations will change throughout the food supply chain. These models have proved useful, for instance, in the design and validation of microbial inactivation treatments (Alvarenga et al., 2022; Peng et al., 2017), shelf life estimation (Rodriguez-Caturla et al., 2023), or risk assessment (Messens et al., 2018, 2017).

One of the most common models is the Baranyi growth model (Baranyi and Roberts, 1994), which is based on the differentical equation shown in Eq. (1).

$$\frac{dN}{dt} = \alpha \cdot \mu \cdot \beta \cdot N \tag{1}$$

It describes the change in the microbial concentration (N) with respect to time (t) using a modification of exponential growth where the maximum specific growth rate (μ) is corrected by two factors: α and β. The factor β introduces the stationary phase as a Verhulst logistic term ($\beta = 1 - N/N_{max}$), whereas α introduces the lag phase. This phase represents a delay in exponential growth, with microbial cells needing some time after inoculation to start dividing, even in conditions that support their growth. This lag phase has been attributed to the need of microbial populations to adapt to the growth conditions (Rolfe et al., 2012).

In the Baranyi model, it is hypothesized that the lag phase is linked to a "theoretical substance" (C) that must be produced to enable growth. The current consensus is to assume that C follows first order kinetics,

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with specific growth rate μ (the same as the microbial population), as shown in Eq. (2).

$$\frac{dC}{dt} = \mu \cdot C \tag{2}$$

Then, the coefficient describing the lag phase is defined as $\alpha = C/(1+C)$. Accordingly, the lag phase duration under constant environmental conditions (λ) is related to the initial value of C (C_0) by Eq. (3) (Baranyi and Roberts, 1994).

$$\lambda = ln\left(1+\frac{1}{C_0}\right)\Big/\mu \tag{3}$$

The Geeraerd model for microbial inactivation (Geeraerd et al., 2000) uses analogous hypotheses as those of the Baranyi growth model. The Geeraerd model is an extension of the first-order inactivation kinetics (Eq. 4) that includes two coefficients (γ and ω) that reduce the specific inactivation rate (k).

$$\frac{dN}{dt} = -\gamma \cdot k \cdot \omega \cdot N \tag{4}$$

The second coefficient $\left(\text{defined as } \omega = 1 - \frac{N_{res}}{N}\right)$ introduces the tail height based on parameter N_{res}. This article focuses in the shoulder length, which is introduced in the Geeraerd model by coefficient γ. The shoulder represents an initial part of the treatment where the microbial concentration remains constant despite the process condition (e.g., treatment temperature) being harsh enough to cause microbial inactivation.

Hence, there is a clear parallelism in the biological interpretation of both the lag phase of growth curves and the shoulder of inactivation curves. This parallelism is also translated in the modelling approaches. In the Geeraerd model, the shoulder is introduced assuming a protective theoretical substance (Q) that must be inactivated before microbial inactivation takes place. In this model, it is assumed that Q also follows first order kinetics with specific rate, k (the same as the microbial population), as shown in Eq. 5.

$$\frac{dQ}{dt} = -k \cdot Q \tag{5}$$

Then, the shoulder length of the population (S_l) is related to the initial value of Q (Q_0) by Eq. (6) (Valdramidis et al., 2005). Although Q_0 can be influenced by empirical factors, such as the come-up time for heating or the protective effect of the media at the beginning of the experiment, it is often assumed to represent the physiological state of the microbial cells.

$$S_l = \frac{ln(Q_0+1)}{k} \tag{6}$$

Despite the Baranyi and Geeraerd models being broadly accepted by predictive microbiologists, there are still some uncertainties in their application. A principal one is the definition of secondary models for the lag phase and the shoulder length (Swinnen et al., 2004). Plenty of empirical evidence obtained under isothermal conditions shows that temperature influences both λ and S_l. This relationship is most often studied by performing various independent experiments at constant temperature. Then, a primary model (both the Baranyi and Geeraerd models have algebraic solutions for isothermal conditions) is fitted to each experiment, obtaining a table of model parameters against storage/treatment temperature. Finally, one secondary models is fitted to the relationship between temperature and k, and an independent secondary model for the relationship between temperature and S_l. Similarly, for growth, independent secondary models are defined for the relationship between temperature and μ, and between temperature and λ. The secondary models for λ are often defined by independent algebraic equations (Delignette-Muller et al., 2005; González-Tejedor et al., 2023). However, some studies have also included the relationship between μ and λ in this second step (Amézquita et al., 2005; Augustin et al., 2000; Jaloustre et al., 2011). This is done by estimating λ from the relationship $\lambda = h_0/\mu$; based on the values of μ estimated from independent experiments.

Although this approach to describe S_l or λ is valid for isothermal conditions, it has important shortcoming for dynamic treatments. Eqs. 3 (for the lag phase) and 6 (for the shoulder length) are only valid for constant environmental conditions. In fact, defining a "shoulder length" or "lag phase duration" for a treatment with varying temperature is illogical, as those apparent phases might be due to the temperature changes (instead of a biological response). Instead, dynamic modelling requires assumptions directly on parameters C_0 (for Baranyi) or Q_0 (for Geeraerd). Both parameters represent a "physiological state" of the microbial cells, standing for the concentration of theoretical substances that must be produced or inactivated before observing changes in the microbial concentration. Therefore, it is reasonable to assume that C_0 or Q_0 depend only on the history of the cell (i.e., under laboratory conditions, the cell preparation method), so they should remain constant for different inactivation or growth experiments (dynamic or isothermal), provided the cell-preparation procedures are the same.

Considering the current limitations regarding the definitions of secondary models for the lag phase and shoulder, this study revisits the Baranyi and Geeraerd models, analysing in detail the implications of the assumption of a constant C_0 or Q_0. We follow a deductive reasoning approach, where we study first in the "Theory" section what secondary model equations are compatible with the dynamic assumptions of the Geeraerd and Baranyi models. Next, using data from the scientific literature, in the "Results and Discussion" section we validate the theoretical analysis and illustrate the implications of the theoretical analysis in terms of robust parameter estimation and for the verification of the hypotheses of the Baranyi and Geeraerd models.

2. Theory

2.1. Determination of the secondary models compatible with the Geeraerd hypotheses

2.1.1. Theorem

Provided the relationship between k and temperature is log-linear, the only secondary model for S_l compatible with a constant Q_0 between experiments in the Geeraerd model is also log-linear.

2.1.2. Proof

It is common to assume in the Geeraerd model that the relationship between k and temperature (T) is log-linear. This is shown in Eq. (7), where a and b are regression coefficients.

$$lnk = a + b \cdot T \tag{7}$$

Considering the identity $k = \ln(10)/D$, this secondary model is equivalent to the Bigelow secondary model for the D-value (D).

Let us assume now that we perform a batch of inactivation experiments under isothermal conditions. In each of them, the relationship between S_l, k and Q_0 will be described by Eq. (6). By substituting the secondary model (Eq. 7) into Eq. (6), we obtain Eq. (8):

$$S_l = \frac{1}{k} ln(Q_0+1) = \frac{1}{e^{a+b\cdot T}} ln(Q_0+1) = e^{-a} e^{-b\cdot T} ln(Q_0+1) \tag{8}$$

As described above, the quantity Q_0 represents a hypothetical physiological state of the cells. Therefore, it is reasonable in the Geeraerd model to assume this parameter to be constant between experiments (as long as the culture preparation method remains unchanged between experiments). This implies that $ln(Q_0+1)$ is also constant, as well all as e^{-a} that is a parameter of the secondary model in Eq. (7). Ergo, by assuming a log-linear secondary model (Eq. 7) and that Q_0 = constant, Eq. (9) is the only secondary model for S_l compatible with the dynamic hypotheses of the Geeraerd model, where $A = e^{-a} ln(Q_0+1)$.

$$Sl = Ae^{-b\cdot T} \tag{9}$$

Or, equivalently,

$$lnS_l = A' - b\cdot T \tag{10}$$

Where $A' = lnA$.

Furthermore, it is worth highlighting that there is a link between this secondary model (Eq. 10) and the one for k (Eq. 7) because coefficients a and b appear in both (i.e., b is the slope of both relationships and a appears within A). Therefore, the secondary model for S_l introduces a single additional unknown: Q_0 (or a transformation of it), so only three unknown parameters are required to describe both secondary models.

2.2. Determination of the secondary models compatible with the Baranyi hypotheses

2.2.1. Theorem

Provided the suboptimal Ratkowsky (square-root) model for μ and temperature, the only secondary model for S_l compatible with a constant Q_0 in the Baranyi model is an inverse T^2 model.

2.2.2. Proof

Although a variety of secondary models have been proposed for μ in the Baranyi model, for simplicity, we limit our analysis to the suboptimal Ratkowsky square-root model (Ratkowsky et al., 1982) shown in Eq. (11). This model introduces two parameters: b_R (the slope of the relationship between temperature and the square root of μ) and T_{min} (the theoretical minimum temperature for growth).

$$\sqrt{\mu} = b_R(T - T_{min}); T > T_{min} \tag{11}$$

$$\sqrt{\mu} = 0; otherwise$$

We now follow a similar approach as in section 2.1 for the Geeraerd model, assuming that a batch of parallel isothermal growth experiments are performed. Accordingly, C_0 would remain constant between experiments (provided the same culture-preparation methods are used).

If we substitute the secondary model (Eq. 11) into the equation that describes the relationship between λ, μ and C_0 (Eq. 3), we obtain Eq. (12):

$$\lambda = \frac{1}{\mu} ln\left(1 + \frac{1}{C_0}\right) = \frac{1}{b_R^2 (T - T_{min})^2} ln\left(1 + \frac{1}{C_0}\right) \tag{12}$$

Using similar arguments as for the Geeraerd model, it is logical to assume in the Baranyi model that the quantity C_0 will remain constant between experiments, as it depends only on the history of the cells. Then, Eq. (12) can be simplified to Eq. (13), where $B = \frac{ln\left(1+\frac{1}{C_0}\right)}{b_R^2}$

$$\lambda = B \frac{1}{(T - T_{min})^2} \tag{13}$$

Or, equivalenty

$$\frac{1}{\sqrt{\lambda}} = \frac{1}{\sqrt{B}} (T - T_{min}) \tag{14}$$

Ergo, considering the secondary model for μ in Eq. (11), the model represented by Eq. (13) (or equivalently Eq. (14)) is the only secondary model for λ compatible with the assumption of a constant C_0. Note that, in a similar way as the Geeraerd model, this model is linked with the one for μ by parameters T_{min} and b_R, so it only introduces an additional unknown: C_0 (or a transformation of it). Hence, both secondary models would be described by three model parameters.

3. Materials and methods

3.1. Microbial inactivation data

The data on the inactivation of *Listeria innocua* in coconut water and acidified Tryptic Soy Broth (TSB) reported by González-Tejedor et al. (2023) was revisited in this study. This dataset was selected due to the presence of clear shoulders, as well as the use of two different media, facilitating the validation of our approach. Briefly, an inoculum of stationary-phase cells of *L. innocua* CECT 910 was prepared using standard incubation methods. The microorganism was inoculated in either coconut water (purchased from a local retailer from Panama) or TSB acidified to pH 5.6 (the one measured on the coconut water) and treated at five different isothermal temperatures (50, 52.5, 55, 57.5 and 60 °C) in a Mastia thermoresistometer (Conesa et al., 2009). The duration of the treatment was adjusted for each condition in order to observe approximately 4 to 5 log-reductions, taking enough intermediate time points to fit a microbial inactivation model.

The data showed a clear shoulder, so the Geeraerd model (without tail) was fitted to the data obtained at each temperature. It was fitted using the online version of *bioinactivation* (Garre et al., 2018, 2017), currently available at https://foodlab-upct.shinyapps.io/bioinactivation4/. For this type of fit, *bioinactivation* uses non-linear regression on the algebraic solution of the Geeraerd model.

In the original study, the authors fitted a Bigelow-type model for the *D*-value and a log-linear secondary model for the shoulder length. They reported the values of S_l and D estimated for each condition, so those values were directly extracted (converting D to k by the identity $k = (\ln 10)/D$) and reanalysed using the approach proposed in section 2.1 of this study. This implies not just the use of the same type of secondary model (log-linear), but also including a link between both secondary models, so there are only three unknown parameters instead of four.

3.2. Microbial growth data

The data on the growth of *Escherichia coli* in/on cheese reported by Kim et al. (2014) was used to evaluate the secondary modelling approach for microbial growth proposed here. This dataset was selected because it includes experiments on four media at four different temperatures, enabling further validation of our approach. Briefly, the authors prepared a cocktail of five *E. coli* strains (NCCP14037, NCCP 14038, NCCP 14039, NCCP 15661, and ATCC11142) in TSB at 35 °C until the cells were in stationary phase. The cocktail was then inoculated on the surface of four different types of cheese (brie, mozzarella, camembert and cheddar) purchased from a local retailer (from Korea).

The samples were stored at different isothermal temperatures (10, 15, 25 and 30 °C), taking between 9 and 16 samples. The microbial concentration on each sample was estimated by serial dilution and plate counts. Although the original study compared different primary models, we extracted only the model parameters (μ and λ) estimated for the Baranyi model for compatibility with our theoretical analysis from section 2.2. These parameter values were directly reported by the authors in the original publication, although the original study reported the growth rate in $\log_{10}$ CFU/h. Hence, it was converted to specific growth rate (ln CFU/h) for compatibility with our approach.

The original study used as secondary model for μ either the suboptimal Ratkowsky model or a second order polynomial model. For λ, it used an inverse square-root model. In our study, according to section 2.2, we used the same type of secondary models, but we also included the link between the both secondary models. Therefore, the number of unknown parameters is only three instead of four like in the approach of the original study.

3.3. Model fitting

The approaches proposed for microbial inactivation (section 2.1) and

microbial growth (section 2.2) link the secondary models for k and S_l, or for μ and λ. As a result, instead of four unknown parameters (as would be the case for two independent models), the parameter estimation is reduced to three parameters.

To properly account for this link, both secondary models were fitted in a single step using weighted least squares. The parameter estimation problem can be written as the optimization problem shown in Eq. (15). It consists of finding the vector of parameters, θ (a, b and C_0 for inactivation; T_{min}, b_R and Q_0 for growth), that minimize the sum of squared residuals. Because two different secondary models are fitted at the same time, the sum of squared residuals consists of two sums. This is indicated by r_1 ($r_1 = lnk_{pred} - lnk_{obs}$ for inactivation; $r_1 = \sqrt{\mu}_{pred} - \sqrt{\mu}_{obs}$ for growth) and r_2 ($r_2 = SL_{pred} - SL_{obs}$ for inactivation; $r_2 = \lambda_{pred} - \lambda_{obs}$ for growth). The weights w_1 and w_2 account for the fact that both variables have different scales and units. They were set to the inverse of the variance of observed parameter values, as suggested in van Boekel (2009), to avoid that one variables dominates the model fit.

$$\underset{\theta}{\operatorname{argmin}} \sum w_1 r_1^2 + \sum w_2 r_2^2 \tag{15}$$

The parameter estimation problem was implemented in R version 4.2.3 (R Core Team, 2022). The optimization problem was solved using version 1.3.6.2 of the *FME* package (Soetaert and Petzoldt, 2010). The goodness of the fit was evaluated using the weighted root mean squared error (*wRMSE*), calculated as shown in Eq. (16) where n is the total number of data points. The code implemented is available from the GitHub page of one of the co-authors (https://github.com/albgarre/revisiting-secondary-models).

$$wRMSE = \sqrt{\frac{(\sum w_1 r_1^2 + \sum w_2 r_2^2)}{n}} \tag{16}$$

4. Results and discussion

4.1. Application to the theoretical analysis to the inactivation of Listeria innocua in TSB and coconut water

The data from González-Tejedor et al. (2023) on the inactivation of *L. innocua* was revisited using the novel secondary modelling approach proposed here. As illustrated in Fig. 1, the fitted models describe the overall trend of the observations for both the shoulder length, S_l, and the inactivation rate, k. This could be expected, as the original authors also used a log-linear secondary model for the D-value (which is equivalent to Eq. 7) and a log-linear secondary model for S_l (equivalent to Eq. 9; see Eq. 10).

Nonetheless, based on the analysis presented here, the models by González-Tejedor et al. (2023) would be overparameterized because that study used two independent secondary models for the inactivation rate and the shoulder length. Each of those models had two parameters, resulting in a total of four unknowns. As concluded in section 2.1, both models are linked, so only three model parameters would be needed to describe the data.

Table 1 reports the parameter values estimated from the data in TSB and in coconut water. Due to the different model hypotheses, the only parameter that is partly comparable to those reported by González-Tejedor et al. (2023) is the slope term b. Nonetheless, our current study fits the model in the scale of the natural logarithm, whereas the previous one by González-Tejedor et al. (2023) used a scale of decimal logarithm for the secondary model. Furthermore, this study fits the secondary model for S_l targeting the residuals of S_l, whereas the other one applied a log-transformation. Despite these differences, the parameters are still similar. The original study reported b values of 0.20 °C^{-1} in TSB and 0.23 °C^{-1} in coconut water. Converting the values in Table 2 to natural logarithm results estimates of 0.16 °C^{-1} and 0.17 °C^{-1}, in TSB and coconut water respectively; a relatively small deviation considering the different methodologies. This provides strong evidence supporting that there is a link between the secondary models for S_l and k. This implies that a majority of previous studies in the field developing inactivation models based on the Geeraerd model (following a methodology similar to González-Tejedor et al. (2023)) would be overparameterized.

4.2. Application to the theoretical analysis to the growth of E. coli on cheese

Using a similar approach as for the inactivation data, the data reported by Kim et al. (2014) was revisited using the secondary models developed here for the Baranyi model, including the link between the

Table 1
Parameters (estimate ± standard error) of the secondary models estimated from the data on inactivation of *Listeria innocua* on TSB and Coconut water.

	a (·)	b (1/ °C)	$\log_{10} Q_0$ (·)	*wRMSE* (·)
TSB	−20.49 ± 1.02	0.37 ± 0.02	2.88 ± 0.35	0.12
Coconut water	−21.97 ± 1.36	0.40 ± 0.02	1.93 ± 0.31	0.14

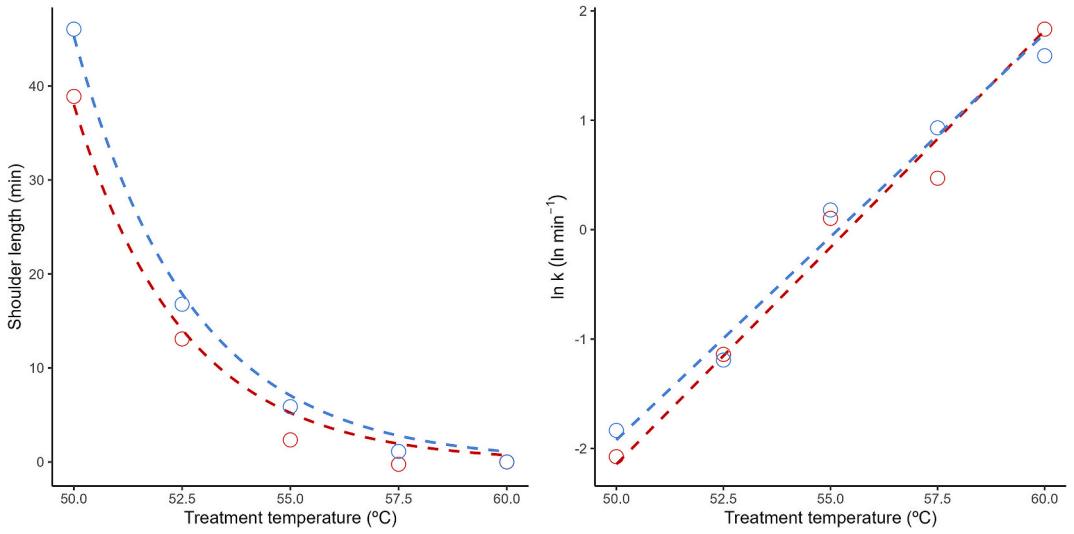

Fig. 1. Fit of the secondary models (Eqs. 8 and 7, respectively) to the parameters of the Geeraerd model fitted to the inactivation of *Listeria innocua* in coconut water (red) and TSB (blue).

Table 2
Parameters (estimate ± standard error) of the secondary models estimated from the data on growth of *Escherichia coli* on four different types of cheese.

	T_{min} (°C)	b_R (h^{-2} °C^{-1})	$\log_{10} C_0$ (·)	*wRMSE* (·)
Brie	1.6 ± 3.1	0.046 ± 0.008	−1.34 ± 0.68	0.39
Camembert	2.9 ± 2.5	0.050 ± 0.007	−1.39 ± 0.71	0.34
Mozzarella	4.9 ± 0.7	0.036 ± 0.002	0.35 ± 0.15	0.11
Cheddar	7.3 ± 0.6	0.036 ± 0.001	−1.15 ± 0.13	0.06

secondary models of μ and λ. Figs. 2 and 3 illustrates the secondary models fitted to parameters λ and μ, respectively, with Table 2 reporting the values of the parameter estimates.

The secondary model for λ was suitable for the description of the lag phase duration in the four media included in the study by Kim et al. (2014), as depicted in Fig. 2. Regarding μ, the secondary model fitted the data well on cheddar and mozzarella cheeses. Accordingly, the parameter reduction concluded in section 2.2 would be suitable for this dataset, evidencing that previous studies that used independent secondary models for λ and μ would be overparameterized.

On the other hand, the secondary models for μ fitted to the data on both brie and camembert cheese clearly underfit the maximum specific growth rate reported by Kim et al. (2014) at 30 °C. This could indicate that the secondary model for μ (Eq. 11) is unsuitable for these particular experiments. Following deductive reasoning, this would imply that the dynamic hypotheses of the Baranyi model (eqs. 1–3) would be unsuitable to describe this growth experiment because the inverse quadratic relationship is a direct conclusion of them that was invalidated by the empirical observations.

However, a closer inspection of the data reported in the original study shows a maximum specific growth rate of 2.16 ln CFU/h and 2.37 ln CFU/h in brie and camembert cheese, respectively. These values could be considered unusually high for *E. coli* when comparing against data from the literature. Namely, the broth models available in *ComBase* for *E. coli* (Baranyi and Tamplin, 2004) calculate $\mu = 1.71$ ln CFU/h for 30 °C and pH 7.0. Considering that these models are built from data intended as a worst-case-scenario (i.e., conditions as favourable as possible for microbial growth for a given pH/temperature combination), it is unusual for experimental data on a food product to report higher values of μ. Ačai et al. (2016) reported values similar to those of *ComBase broth models* for *E. coli* in milk (1.3 ln CFU/h). Data contained in ComBase reported by the Institute of Food Research for *E. coli* in broth at 30 °C (entries B302_57 and B302_93) and from the scientific literature (Gill and Phillips, 1985) all estimate values of μ close to 1.7 ln CFU/h, all of them much lower than those reported by Kim et al. (2014). This may indicate that, instead of Eq. (11) being unsuitable for this experiment, the discrepancy is due to some overestimation in the values reported in that study. Nevertheless, the reasons for that overestimation could not be investigated further as the raw data from Kim et al. (2014) is unavailable.

As illustrated in Fig. 3, the secondary models fitted to the growth parameters reported on brie and camembert cheese estimate a value of $\mu = 1.6$ ln CFU/h for a storage temperature of 30 °C. Interestingly, this value is in line with the empirical data from the scientific literature referred above. This could be due to the link introduced in our approach between the secondary models of μ and λ. It is reasonable to assume that this constraint would results in more robust parameter estimates, as a deviation in one parameter estimate (e.g., μ or k) could be compensated by a correct estimate of the other parameter (e.g., λ or S_l) at the same temperature. Therefore, the approach deducted here, would result in generally more robust secondary models for microbial growth.

It is worth mentioning that a principal limitation of our study is the use of a two-step fitting approach between the primary and secondary models, so the uncertainty in parameter estimates of the primary model is not considered in the estimation of the secondary models. Therefore, combining this approach with the fitting of the primary model using a one-step regression analysis presents a clear way to further improve the methodology (Valdramidis et al., 2008). This would require including the linked secondary models in the definition of the primary models, estimating every parameter at once, a methodology that is often more robust (van Boekel and Zwietering, 2007). Although from a computational point of view that step is relatively simple, one-step approaches also have some practical limitations. For instance, they do not explicit demonstrate whether the secondary model fits well the parameters of the primary model (μ,λ,k,S_l). As a result, microbiological factors such as

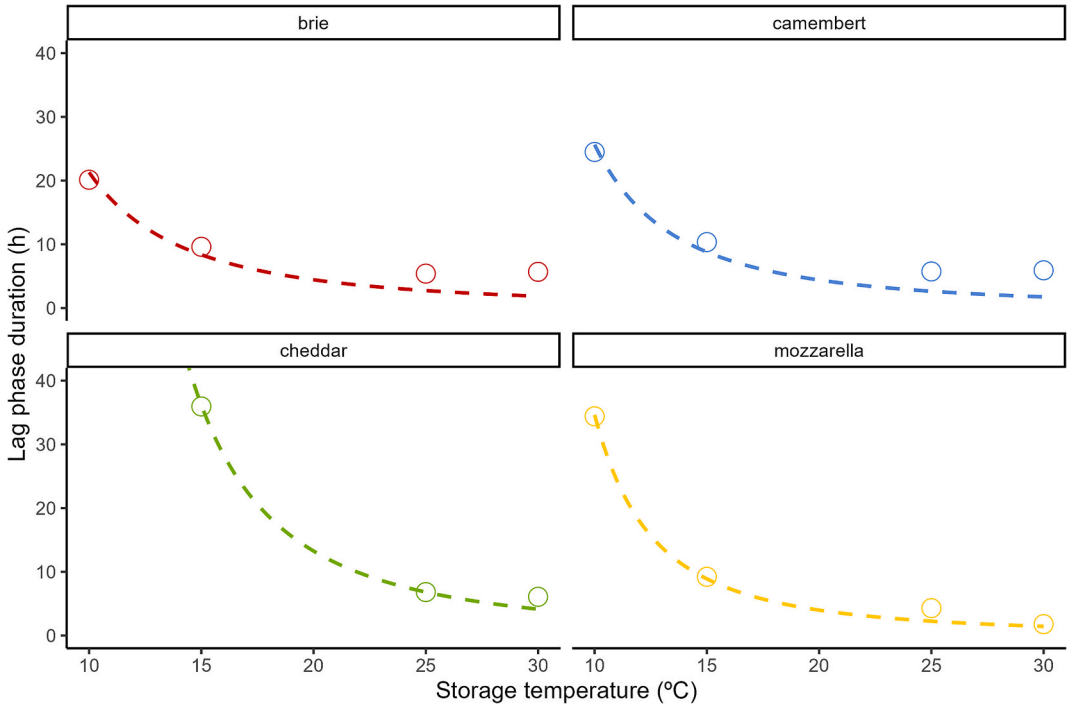

Fig. 2. Fit of the secondary models (Eq. 13) to the lag phase duration (λ) of the Baranyi model for the growth of *Escherichia coli* on four different types of cheese.

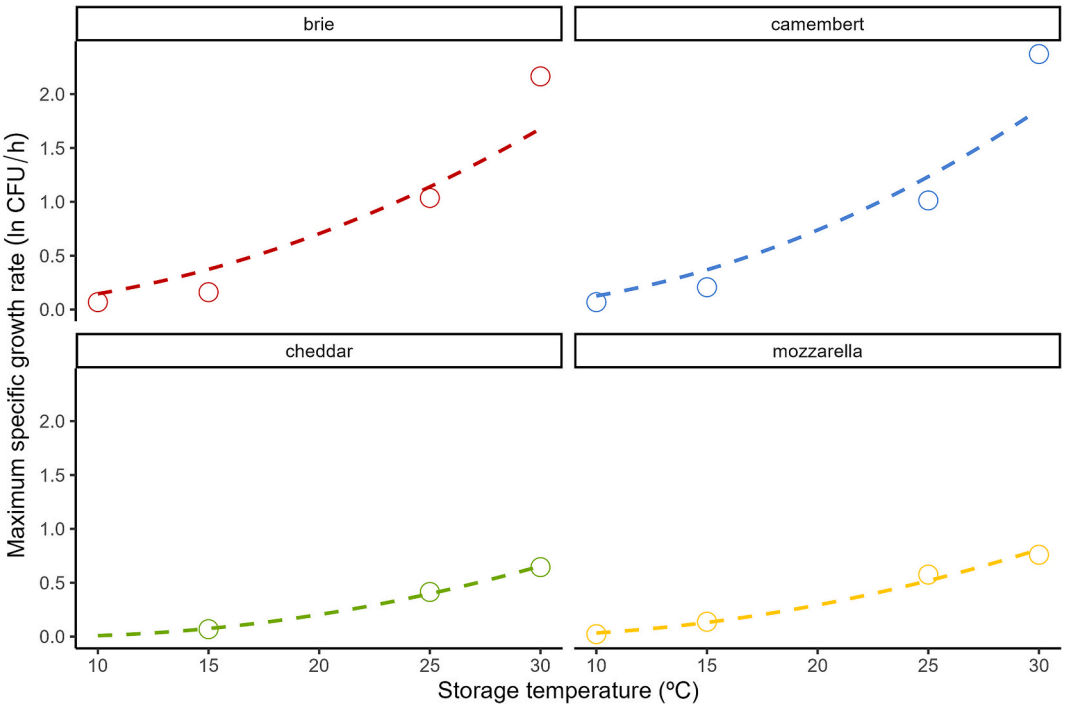

Fig. 3. Fit of the secondary models (Eq. 13) to the specific growth rate (μ) of the Baranyi model for the growth of *Escherichia coli* on four different types of cheese.

the variability in lag/shoulder times or variability in the media can introduce artefacts in the model that are harder to identify in a one-step approach than using the classical two-step method (Georgalis et al., 2023).

Another limitation of our study is the restriction to the temperature effect. One of the goals of the study was to show the parallelism between the shoulder of the Geeraerd model and the lag phase of the Baranyi model. It is generally accepted that temperature has a similar effect on μ (for suboptimal conditions) and k (one that can be linearized), facilitating that illustration. Although the effect of other factors (e.g., pH or water activity) has not been studied on such detail, it is expected to be non-linear and different between growth and inactivation. Therefore, including additional factors would not show the parallelism between growth and inactivation with such clarity, so their inclusion is left for future studies that are based on the novel concepts and methodologies proposed here.

As mentioned in the introduction, our approach for growth modelling has some similarities with previous studies that estimated λ from the relationship $\lambda = \boldsymbol{h_0/\mu}$ (Amézquita et al., 2005; Augustin et al., 2000; Jaloustre et al., 2011). However, there is a fundamental difference. Here, we follow deductive reasoning to demonstrates the link between the secondary models for both parameters, enabling a simultaneous estimation of both secondary models (Eq. 11 and 14). This is not based on any empirical observation, but on an analysis of the model hypotheses that is later validated using empirical data. Therefore, it should be considered more robust than previous studies that had a stronger empirical basis.

5. Conclusions

We demonstrate that the dynamic hypotheses of the Baranyi and Geeraerd model impose resctrictions on the validity of secondary models: a log-linear secondary model between temperature and S_l for Geeraerd, and an inverse quadratic relationship between λ and temperature for Baranyi with a suboptimal Ratkowsky secondary model for μ. Furthermore, the secondary models between S_l and k (Geeraerd) and λ and μ (Baranyi) are coupled, reducing the number of unknown parameters by one (i.e., three in total). This result is of high importance for predictive microbiology because it resolves the uncertainty in the definition of secondary models for these parameters, providing clear rules on what model equations can be chosen. This will improve the robustness of predictive microbiology models, especially due to the reduction in the number of parameters from four to three. Also, due to the identification of a close link between isothermal and dynamic conditions, it enables the use of isothermal experiments to check the validity of the hypotheses of the Baranyi and Geeraerd model (related to a theoretical substance), something that is practically impossible using dynamic data. Despite being limited to the effect of temperature, the general approach and methodology are also applicable to other type of secondary models, including both models with different equations and models including additional environmental factors. Therefore, this article serves as a blueprint for future studies that apply this methodology to similar case studies.

CRediT authorship contribution statement

Alberto Garre: Writing – review & editing, Writing – original draft, Visualization, Software, Funding acquisition, Formal analysis, Conceptualization. **Vasilis Valdramidis:** Writing – review & editing, Validation, Supervision, Investigation, Formal analysis. **Silvia Guillén:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The authors do not have permission to share data.

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