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Cardinal temperatures of *Bacillus licheniformis* growing in various plant-based milk-alternatives

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ABSTRACT

We demonstrate that the commonly assumed matrix-independence of the cardinal (minimum, optimum and maximum) temperatures for bacterial growth is not necessarily valid for *Bacillus licheniformis* growing in plant-based milk. If confirmed, a consequence is that the ratio (called correction factor) between the maximum specific growth rate in a specific food matrix and in culture medium is not temperature-independent for every food matrix, opposed to general expectations.

We found that, while the cardinal temperatures of *B. licheniformis* growing in either white almond or coconut beverages did not significantly differ from those in culture medium, this was not the case for another almond-based beverage, where we observed a smaller growth range and lower optimum temperature. A possible reason for this is that the food composition affects the cardinal temperatures.

Our investigation is an example of "tertiary modelling" inasmuch it studies the effect of food matrix (a category variable) on the parameters of secondary models.

1. Introduction

The plant-based beverage market has grown rapidly, driven by increased consumer awareness of health, environmental sustainability, and ethical concerns regarding animal-based foods (Rime, 2020; Sethi et al., 2016). Plant-based milks derived from oats, almonds, soy, and coconut are now widely available and commonly marketed as lactose-free, cholesterol-free, vegan-friendly, and low in saturated fats (Giugliano et al., 2023; Kain et al., 2024).

Among microorganisms of concern in such foods is *Bacillus licheniformis*, a gram-positive, spore-forming, moderate thermophilic/thermotolerant bacterium. Its occurrence in food, particularly in dairy, is due to contamination from environmental sources like soil, silage, and farm environments, as well as biofilms in processing factories (Fan et al., 2024; Gopal et al., 2015). *B. licheniformis* has been found to contribute to food spoilage through enzymatic degradation, off-flavors, and production of slimy substances that can significantly affect food quality (Lücking et al., 2013; De Jonghe et al., 2010). Although not normally included in the leading foodborne pathogens, it may cause foodborne illness via toxin production under specific conditions (Yeak et al., 2022; Salkinoja-Salonen et al., 1999). It is also known for its heat-resistant spores which are difficult to inactivate with standard pasteurization or cleaning techniques (Setlow and Johnson, 2019; Setlow, 2006).

B. licheniformis poses several challenges to the food business operators, particularly those producing plant-based milk alternatives, due to its ability to (1) grow in harsh conditions thus causing spoilage; (2) form heat resistant spores; and (3) form biofilms (Kyrylenko et al., 2023). To address those challenges, a detailed analysis of the kinetics of *B. licheniformis* in plant-based milk alternatives is vital.

Predictive microbiology provides powerful tools to analyze microbial behavior in food systems as a response to environmental conditions such as temperature, pH, and water activity (McMeekin et al., 2002). To predict the growth of microorganisms in food, experiments are traditionally conducted at various constant temperatures, using a laboratory culture medium. The variation of the natural logarithm of the bacterial concentration over time is used to determine, by a primary model, the maximum specific growth rate, μ_{max}, for each tested temperature. This μ_{max} growth parameter is then considered as a function of temperature (secondary model), for which the minimum, optimum and maximum (i.e. *cardinal*) temperatures, denoted by T_{min}, T_{opt}, T_{max}, are frequently used

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as parameters (Rosso et al., 1993). Based on the assumption that these cardinal temperatures are matrix-independent, predictions on the maximum specific growth rate in food can then be generated via a correction factor typical of that food (Buss da Silva et al., 2017). Under the above assumptions, a correction factor c_f is basically the ratio between the optimum maximum specific growth rate in the food in question and that in culture medium broth:

$$c_f(food) = \mu_{opt.food} \Big/ \mu_{opt.broth} \tag{1}$$

The assumption that the maximum specific growth rate in food differs from that in culture medium only by a correction factor, a temperature-independent constant, is convenient and has been validated on various food products (Misiou et al., 2023; Martinez-Rios et al., 2019; Zhao et al., 2014). However, to our knowledge, no systematic study has been published yet whether this simplification is valid for any food matrices. Such an investigation would be especially timely, as it has long been known that for some thermophilic bacteria, T_{max} for example does depend on the substrate (Merkel and Perry, 1977).

This study aims to develop and evaluate a growth model for *Bacillus licheniformis* in plant-based milk alternatives. In addition, we assess the reliance on the cardinal temperatures and the c_f correction factor when predicting the growth of this organism in three different plant-based milk alternatives. We also examine how the introduction of the logarithm link function for the maximum specific growth rate affects the performance of the regression when fitting secondary models in line with ISO-2025 recommendations.

2. Material and method

We divide the methods into *"wet"* and *"dry"* following the terminology of Rockaya and Baranyi (2025).

2.1. *"Wet" methods*

2.1.1. *Plant-based milks characterization*

To capture the variability caused by the food matrix, we used three plant-based milk alternatives available in the Hungarian market: a white almond milk (A) and a coconut milk (C) from the same company (A) (so the products are named 'AA' and 'CA' respectively), as well as another almond milk from a different company (H) named 'AH'. Brain Heart Infusion 'BHI' (VWR Chemicals) was used for culture medium, as a base for comparison.

For each matrix, macronutrient, pH and water activity measurements were performed using Kjeldahl method for protein, phenol-sulfuric acid titration for carbohydrates, Soxtec extraction for fat, a pH meter with a glass electrode (SevenEasy, Mettler Toledo) for pH and a water-activity meter (rotronic HygroLab C1) for a_w.

2.1.2. *Inoculum preparation*

B. licheniformis DSM 13 reference strain (Leibniz Institute DSMZ) was used in this study. A working culture of the strain was produced and maintained at -20 °C on Microbank™ cryobeads (Pro-Lab Diagnostics) prior to use. The culture of the strain was prepared by inoculating 10 mL of BHI broth with a bead from our working culture and incubating it at 37 °C (ESCO) while shaking under aerobic conditions for 8 h. After the incubation period, 100 μL of the subculture was transferred to a tube containing 9.9 mL of BHI broth and incubated for another 8 h under the same conditions. For all experiments the strain was harvested at the end of the exponential phase and at the beginning of its stationary phase.

2.1.3. *Growth experiments*

For each experiment, a new inoculum was prepared as described above. It was then decimally diluted four times in Tryptone salt diluent (NutriSelect® Basic, Millipore), and 100 μL of the diluted subculture was then inoculated into a 100 mL Erlenmeyer flask containing 70 mL of sample medium (AH, AA, CA, BHI) to reach a target initial concentration around 10^2 CFU/mL. For the 'AH' and 'BHI' media, triplicates were prepared at all temperatures. For the 'AA' and 'CA' media, triplicates were prepared at cold temperatures but only duplicates at high temperatures.

For each experiment, the inoculated samples were incubated at the selected temperatures (ranging from 15 to 55 °C, see Section 2.1.4) while shaking under aerobic conditions.

To enumerate the variation of *B. licheniformis* concentration over time, plate counting method was used by collecting 1 mL of each matrix, adopting the adequate dilutions before pour-plating on BHI Agar (Liofilchem).

Pour-plating was chosen because, under aerobic conditions, *B. licheniformis* rapidly spreads across the surface of the Petri dish, making colony counting difficult. To mitigate this, plates were incubated at 37 °C for 20 h (shorter than the standard 24 h) for easier counting, and colonies were then counted manually.

For each experiment, we aimed to take regular samples for plate counting, acquiring a minimum of 3 points in the lag phase, 6 points in the exponential phase, and 3 points in the stationary phase.

2.1.4. *Choosing the temperature levels*

Growth experiments were carried out at **13** different temperatures (15, 17, 19, 21, 29, 33, 37, 39, 45, 49, 51, 53, 55 °C) in BHI and **12** temperatures (15, 17, 19, 21, 29, 35, 39, 45, 49, 51, 53, 55 °C) in almond milk (AH). For white almond milk (AA), and coconut milk (CA) the experiment was carried out in the same temperature range, at **9** temperature points (15, 17, 19, 21, 33, 37, 45, 51, 55 °C).

2.2. *"Dry" methods*

2.2.1. *Primary modelling*

Primary growth models describe the change of microbial population over time under constant environmental conditions. We fitted the algebraic solution of the model of Baranyi and Roberts (1994) Eqs. (2a) & (2b) to the Ln(cell-concentration) data and thus estimated the specific growth rate (μ_{max}) values at each studied temperature:

$$y(t) = y_0 + \mu_{max}A(t) - \frac{1}{m}Ln\left(1 + \frac{e^{(m\cdot\mu_{max}\cdot A(t))-1}}{e^{(m\cdot y_{span})}}\right) \tag{2a}$$

$$A(t) = t - \lambda + \frac{1}{n\cdot\mu_{max}}Ln\left(1 - e^{(-n\cdot\mu_{max}\cdot t)} + e^{(-n\cdot\mu_{max}\cdot(t-\lambda))}\right) \tag{2b}$$

where y(t) is the natural logarithm of the cell concentration (CFU/mL) at the time t; μ_{max} is the maximum specific growth rate of the cell population (1/h), estimated by the maximum slope of the fitted curve; y_0 and y_{max} are, respectively, the natural logarithm of the inoculum and the maximum cell density (CFU/mL); λ is the lag parameter (h); n and m are smoothness-characterizing tuning- (or curvature-) parameters.

2.2.2. *Secondary modelling*

Secondary models describe how the parameters of a primary model change as a function of environmental conditions (temperature, in our case). The μ_{max}-estimates obtained from the primary fitting (see Section 2.2.1) were regressed against temperature using the Cardinal Temperature model of Rosso et al. (1993; see Eq. (3)).

$$\mu = \frac{\mu_{opt}(T - T_{max})(T - T_{min})^2}{(T_{opt} - T_{min})\cdot\left[(T_{opt} - T_{min})(T - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_{min} - 2T)\right]} \tag{3}$$

The model has four parameters, the minimum T_{min}, optimum T_{opt} and maximum T_{max} growth temperatures, and μ_{opt} (the specific growth rate at T_{opt}). This model has been used by many authors because it is easy to assign microbiological meaning to its parameters.

2.2.3. Tertiary modelling

Tertiary models used to be defined as user-friendly computational systems that integrate primary and secondary models into interactive software tools (Whiting, 1995). However, Baranyi et al. (2017) argued that, logically, a tertiary model should describe how the parameters of the secondary model depend on factors like the food matrix and/or the bacterial species. In what follows we use the latter terminology.

2.2.4. Model fitting and statistical analysis

The fitting of both primary and secondary models was performed using an in-house Visual Basic for Applications (VBA) Excel Add-In that implemented the Levenberg–Marquardt algorithm to carry out the non-linear regression i.e. to minimize the residual sum of squares, which is the core of the standard least-squares method.

To evaluate whether the cardinal parameters (μ_{opt}, T_{min}, T_{opt}, T_{max}) differed significantly across matrices, pairwise Welch's test Eq. (4) were performed using the parameter estimates and their standard errors (Welch, 1947):

$$t_{welch} = \frac{Estimate_A - Estimate_B}{\sqrt{SE^2{}_A + SE^2{}_B}} \tag{4}$$

where:

- $\mathrm{Estimate_A}$, $\mathrm{Estimate_B}$: Estimates for the two matrices A and B;
- $\mathrm{SE_A}$, $\mathrm{SE_B}$: respective standard errors of the estimates.

The degree of freedom was calculated using the Welch–Satterthwaite equation (see Eq. (5)) to account for unequal variances.

$$df = \frac{\left(\frac{SE^2{}_A}{n_A} + \frac{SE^2{}_B}{n_B}\right)^2}{\frac{\left(\frac{SE^2{}_A}{n_A}\right)^2}{n_A - 1} + \frac{\left(\frac{SE^2{}_B}{n_B}\right)^2}{n_B - 1}} \tag{5}$$

The significance of the difference was assessed via the calculated t_{welch}-values and their corresponding p-values. Differences with $p < 0.05$ were considered statistically significant at the 95 % confidence level.

3. Results

3.1. Matrix characterization

Among the tested matrices, BHI had the highest protein content (2.42 g/100 mL) and the lowest fat, while AH was the only matrix with clearly measurable carbohydrates and sugars (2.05 and 1.98 g/100 mL, respectively). AA had the highest total fat, and CA stood out for its high saturated fat content which reflects the typical fatty acid profile of coconut products. pH and water activity values were similar across matrices, with all falling within optimal ranges for microbial growth; therefore, their effect was not modeled (Table 1).

We observed other matrix-effects too, including protein precipitation at elevated temperatures in AH during the stationary phase. This may result from protein denaturation or the production of the biosurfactant lichenysin, which is known to destabilize emulsions and to chelate calcium ions (Yeak et al., 2022). Additionally, fat solidification (e.g., of lauric acid) was also detected at low temperatures in 'CA'. However, neither of these appeared to significantly impact microbial growth kinetics.

Note that the pH, in all temperatures and media, was checked before and after the experiment and no significant change was observed.

3.2. Primary fitting and model performance

Plate count data (CFU/mL) as a function of time, collected from experiments in BHI and the three plant-based milk alternatives (AH, AA, CA) matrices across all temperature levels (Section 2.1), were log-transformed to Ln(CFU/mL) and fitted using the model of Baranyi and Roberts (1994).

Two representative examples of one-replicate primary fitting for the four studied matrices are shown in Fig. 1a–b.

Table 1
Energy, macronutrient, pH[a] and water activity averages of each matrix.

in 100 mL product	Almond milk (AH)	Almond milk (AA)	Coconut milk (CA)	BHI
Energy (kcal)	20.20	16.80	9.98	12.00
Fat (g)	0.569	1.140	0.415	0.020
Of which saturated fatty acids (g)	0.069	0.133	0.385	0.012
Carbohydrate (g)	2.05	<0.10	<0.10	0.20
Of which sugars (g)	1.98	<0.10	<0.10	0.20
Protein (g)	0.993	0.747	0.496	2.42
Salt (g)	<0.1	<0.1	<0.1	<0.1
pH	7.62 (0.06)	7.38 (0.07)	7.32 (0.05)	7.4 (0.2)
aw	0.958	0.959	0.956	0.992

[a] The numbers between parentheses are the standard deviations.

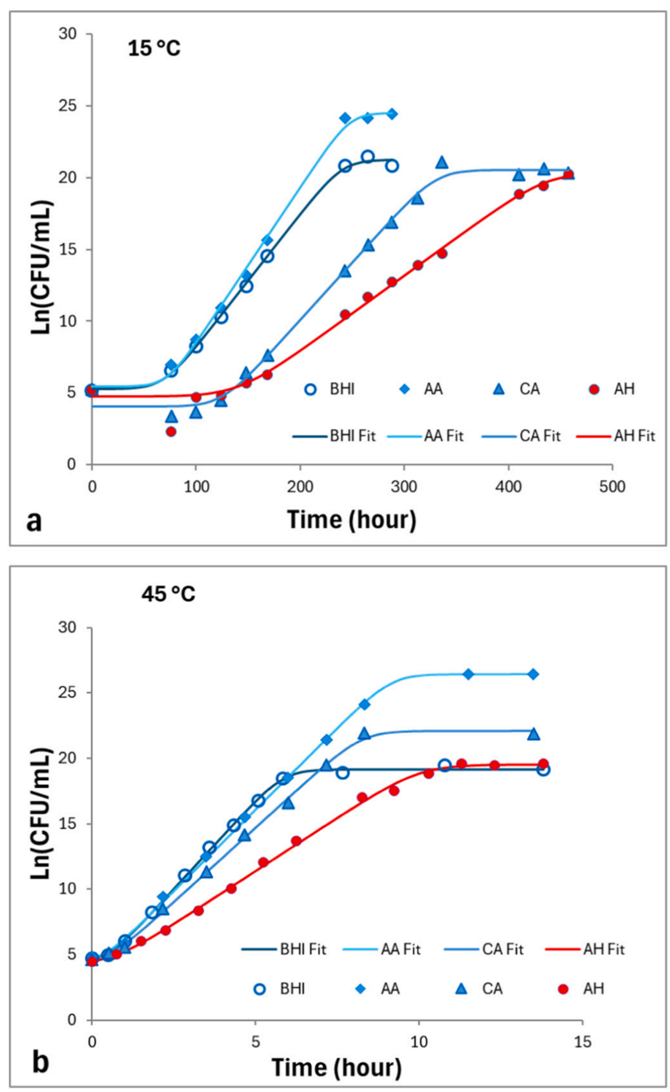

Fig. 1. a–b. Log-counts data in various plant-based matrices at 15 °C (a) and 45 °C (b), fitted by the model of Baranyi and Roberts (1994).

The parameter estimates along with their standard errors and other statistical indicators from the primary modelling are given in the Supplementary Information.

Although our primary focus is the maximum specific growth rate (μ_{max}), it is still worth mentioning that the maximum cell density (y_{max}) also varied with both medium and temperatures, with white almond milk consistently exhibiting the highest values.

Unlike the similar, fast growth observed at 45 °C (Fig. 1b), the growth was slow at 15 °C (Fig. 1a), with average lag times of 68.8 h in BHI and 69.9 h in white almond milk.

In almond and coconut milk, the population underwent a sudden decline immediately after inoculation, a behavior called the "phoenix phenomenon" (Aspridou et al., 2019), resulting in lag phases of 141.2 h and 129.2 h, respectively, before exponential growth resumed. Notably, the same decline-recovery behavior was observed at 55 °C but this time in all the samples, among them the BHI broth, suggesting that both 15 °C and 55 °C lie near the biological limits of growth.

The primary fits (for the 4 matrices and at all temperatures) yielded SE < 0.2 log(CFU) standard-error-of-fit and $R^2 > 99$ % for the goodness of fit, indicating a good accuracy for the original log-count measurements.

More importantly, the data showed that the relative error (in other words, the Coefficient of Variation, CV) of the maximum specific growth rate estimates (Eq. (6)) did not show correlation with the temperature ($p = 0.576$; see Fig. 2).

$$\mathrm{RE}(\mu_{\mathrm{max}}) = \mathrm{SE}(\mu_{max})/\mu_{max} \tag{6}$$

As Rockaya and Baranyi (2025) pointed out, this is a consequence of the dilution-based method used to estimate the original cell concentration, for which the logarithm transformation stabilizes its variance. Similarly, the fact that the relative errors for the μ_{max} estimates vary around a constant, justifies the claim that the error of $\mathrm{Ln}(\mu_{max})$ should be constant. Indeed, when the $\mathrm{SE}(\mu_{max})$ values are plotted against the respective μ_{max} estimates, a clear linear relationship emerged (Eq. (7)).

$$\mathrm{SE}(\mu_{max}) = c \cdot \mu_{max} \tag{7}$$

The estimated c slope corresponds to a certain mid-value of the relative errors of the μ_{max} estimates.

Remember that the approximation, that the errors of the $\mathrm{Ln}(\mu_{max})$ estimates are close to the relative errors of the μ_{max} estimates comes from Eqs. (8a) and (8b):

$$\frac{\mu_{obs} - \mu}{\mu} = \varepsilon \Longrightarrow \mu_{obs} = \mu\,(1 + \varepsilon\,) \tag{8a}$$

$$Ln(\mu_{obs}) = Ln(\mu) + Ln(1 + \varepsilon\,) \approx Ln(\mu) + \varepsilon \tag{8b}$$

Fig. 2 shows that the relative error (RE) does not show a trend with the temperature ($p >> 0.05$). Notably, between 25 °C and 45 °C, the RE values are rather small (2–6 %), corresponding to the "happy-growth region" described by Baranyi et al. (2024). However, RE increases below 20 °C and above 50 °C as the "wet"/measurement uncertainty increases close to the growth/no-growth boundaries. Such pattern was previously reported in other food systems, too (Le Marc et al., 2005).

The resultant ca. 5 % RE for the specific growth rate estimates in our experiments represents high accuracy and significant improvement compared to its typical values (~10 %) generated by other food-based growth experiments (e.g. Pin et al., 2004).

Examples for culture-based growth experiments for *B. licheniformis* can be found in *ComBase* (Baranyi and Tamplin, 2004). There, 48 growth curves (see the records with *ComBase* ID starting with B227, B266, B267) provided the base for its predictive model. These were generated at the same laboratory, under standardized protocol and in culture medium broth; what is more, in a smaller temperature range than the one we use here. Still the relative error of their maximum specific growth rate estimates is around 20 %. The high accuracy of our maximum specific growth rate estimates plays a critical role in the claim that the cardinal temperatures are not the same for the almond-based milk and for the culture medium.

The performance of our model exceeded the ISO guidelines (ISO 23691, 2025), which recommend a Relative Error (RE) below 10 % for maximum specific growth rate estimates and a Coefficient of Variation (CV) below 15 % when three replicates are used. In our study, the RE

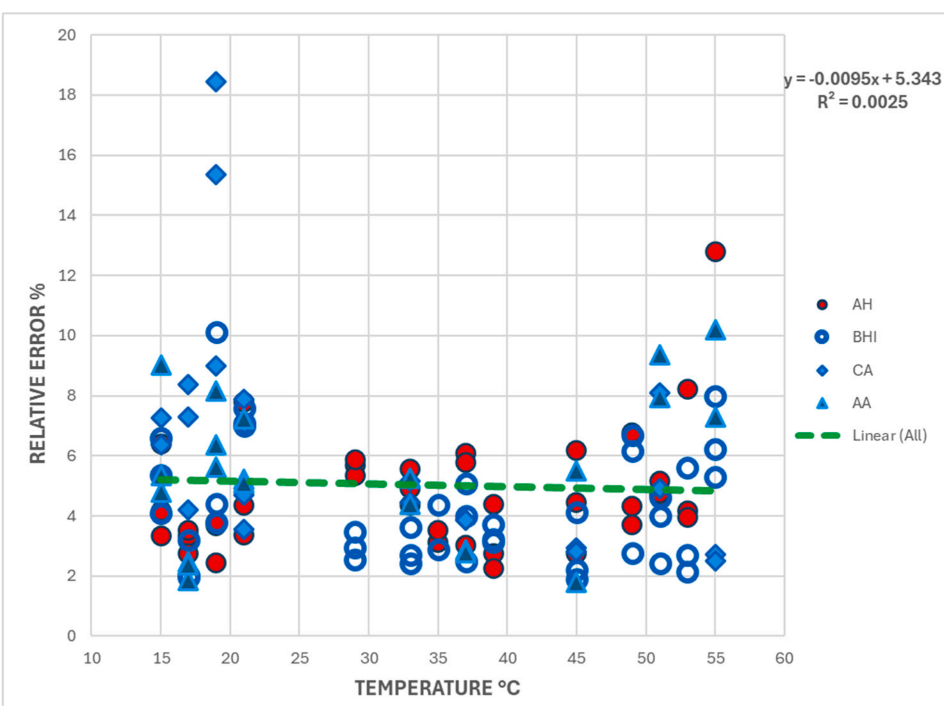

Fig. 2. Relative errors of the maximum specific growth rate estimates, generated by the primary model fitting, against temperature, through all the four studied media.

was below 15 % for all data points, except for two values in the coconut matrix at 19 °C, which showed slightly elevated RE-s (15.3 % and 18.4 %; see Fig. 2). Across all conditions where replicates were available, the CV of μ_{max} remained below 15 %, with an overall average of 4.02 %, indicating a high precision of the growth rate estimations.

3.3. Secondary fitting and model performance

When we fitted the CTM model to our specific growth rate estimates, the standard error-of-fit was 0.10 on the Ln(μ_{max}) scale. This value shows higher accuracy than those characterizing compatible secondary models on the entire (T_{min}, T_{max}) interval. The reason why the logarithm link function should be used for the μ_{max} response variable was analyzed by Akkermans et al., 2018, also confirmed by Rockaya and Baranyi (2025).

The temperature dependence of Ln(μ_{max}) was modeled separately for each matrix using the Cardinal Temperature model (CTM). Fig. 3a and b demonstrates that the model describes the μ_{max} vs. temperature relationship satisfactorily.

However, beyond the goodness of fit, it is equally important to consider the standard errors of the model parameters, which play a crucial role in comparing the models.

Table 2 lists the estimated cardinal parameters, i.e. μ_{opt}, T_{min}, T_{opt} and T_{max}, along with their respective standard errors.

The Cardinal Temperatures of *B. licheniformis* growing in the white almond milk (AA) and the coconut milk (CA) were similar to those in BHI, though the μ_{opt} estimates showed the trend: μ_{opt}(BHI) > μ_{opt}(AA) > μ_{opt}(CA).

An interesting and unexpected difference was observed in the

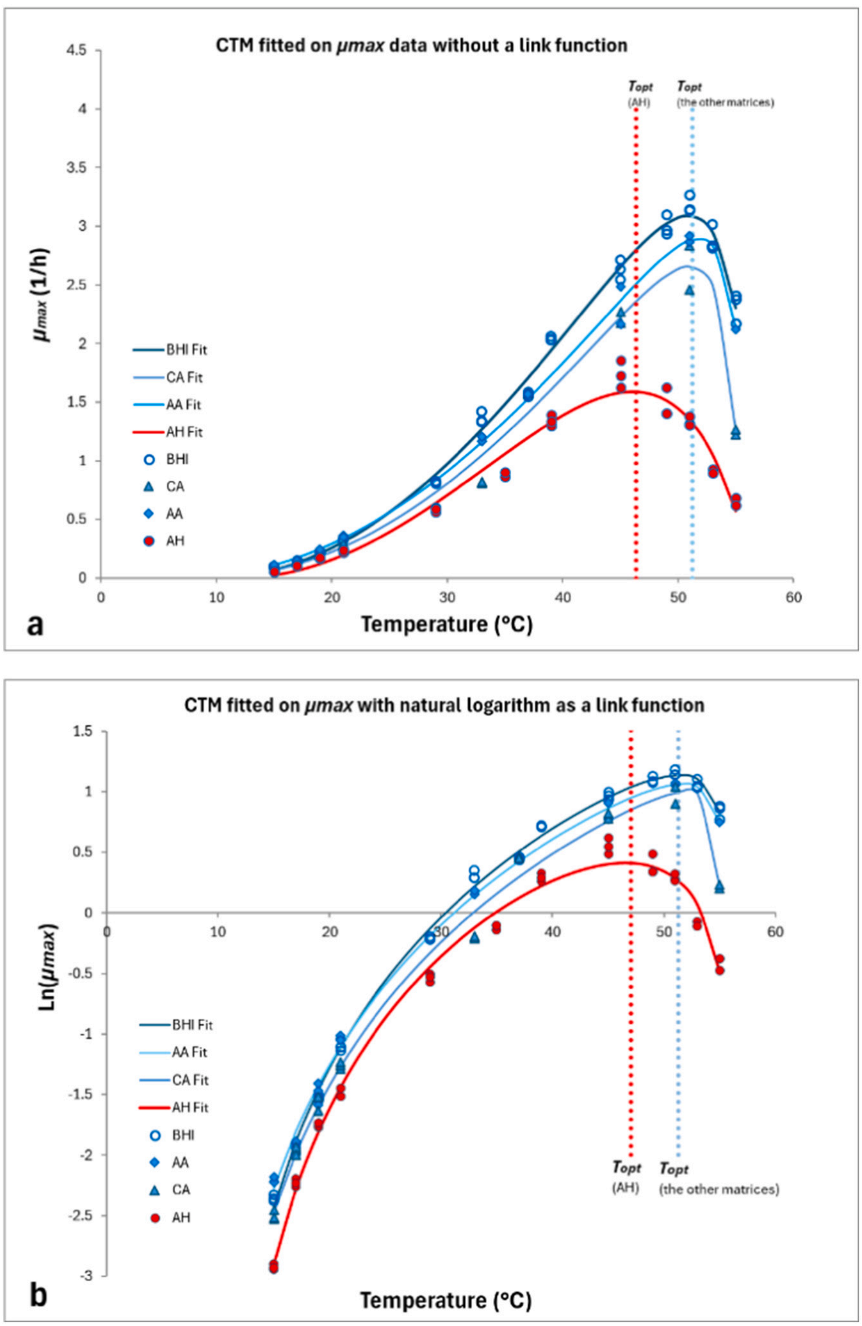

Fig. 3. Specific growth rates (μ_{max}) as a function of temperature fitted by CTM. (a) μ_{max} estimates from the primary model fitting and their fitted CTM curves, without using a link function and (b) the same data but with the natural logarithm link function.

Table 2
Estimated parameters and their standard errors for the CTM model in BHI and the three studied plant-based milks, resulting from the regression while applying the Ln link function.

	T_{min}	SE_{Tmin}	μ_{opt}	$SE_{\mu opt}$	T_{opt}	SE_{Topt}	T_{max}	SE_{Tmax}	SE_{fit}
BHI (n = 39) (L = 13)	8.59	0.16	3.10	0.06	51.36	0.25	56.47	0.33	0.08
Almond milk (AH) (n = 32) (L = 12)	9.60	0.27	1.51	0.03	46.55	0.38	56.74	0.39	0.09
White almond milk (AA) (n = 21) (L = 9)	7.47	0.31	2.89	0.15	51.63	0.62	56.20	0.65	0.06
Coconut milk (CA) (n = 22) (L = 9)	7.76	0.37	2.71	0.24	52.01	1.22	55.29	0.49	0.11

n = number of fitted μ_{max}; L = number of temperature levels.

parameters of almond milk (AH) compared to those of the other plant-based matrices. The well-known delta-shaped curve shifted on the x-axis resulting in higher T_{min} and T_{max} and a very different T_{opt} compared to its values in the other media (Fig. 3a–b). Besides, almond milk (AH) had the smallest μ_{opt} value.

To support the results statistically, pairwise Welch-test was carried out to compare the cardinal parameters across all the media. It showed that the cardinal parameters for almond milk (AH) differed significantly from the parameters of the other matrices (culture medium 'BHI'; coconut 'CA', and white almond 'AA') in all parameters ($p < 0.05$) except T_{max}. In contrast, no significant differences were observed between the cardinal values in BHI, coconut, and white almond matrices ($p >> 0.05$).

Fig. 4 compares observed and predicted values of the natural logarithm of the maximum specific growth rate, Ln(μ_{max}), across three food matrices (AA, CA, AH) using correction factors with the predictions in BHI as a baseline. The equality line represents perfect agreement between predicted and observed values. While predictions in AA and CA show good alignment with the equality line, AH-predictions exhibits notable deviations, particularly at lower growth rates. This indicates that the correction factor, i.e. the ratio μ_{food}/μ_{BHI} is not necessarily temperature-independent for every food matrix, as AH is a counter-example.

Notably, the purple AA points closely follow the equality line, indicating that growth kinetics in AA is similar to that in BHI. Meanwhile, for lower values of Ln(μ_{max}) (below 0.2 h^{-1}), the blue CA points run roughly parallel to the equality line. This shows that a correction factor can reliably adjust BHI-based predictions for CA in this lower range of Ln (μ_{max}). However, the AH points (red) increasingly deviate from the equality line as Ln(μ_{max}) decreases. This indicates that a constant correction factor is inadequate for predictions in AH. These patterns suggest three distinct behaviors in the sub-optimal region: (1) AA behaves like BHI almost without adjustment; (2) CA aligns with BHI after applying C_f; and (3) AH exhibits more complex deviation, where the constant correction factor method is not valid for this matrix.

The performance of the model was evaluated according to ISO guidelines (ISO 23691, 2025), which recommend a Relative Error (RE) below 10 % for μ_{opt} and 1 °C SE for T_{min}. In our study, the RE for (μ_{opt}) estimate was below 10 %, and the SE of T_{min} was <0.5 °C for all matrices.

4. Discussion

Our study demonstrated that the maximum specific growth rate of the *B. licheniformis* growing in AA and CA is just a constant proportion of the rate predicted for BHI, but this does not hold for AH, with distinctly lower T_{opt} optimum temperature. The μ_{opt} optimum specific growth rate was also significantly lower for AH than for the other two food matrices. One reason could be the difference between the beverages in terms of their compositional profile.

AH and AA were derived from the same raw material (almond), but they came from different manufacturers so their formulation and processing were not the same, which might have caused the difference in their microbial kinetics. Remember that unlike bovine milk, which has a relatively standardized composition, plant-based alternatives are human-formulated products with highly variable ingredient profiles and processing methods. Therefore, their source material (e.g., almond, soy, oat) is not necessarily a significant factor to create groups based on their microbial kinetics. An example for their compositional difference is shown by their total carbohydrate content. There is a significant negative correlation (slope = –1.73, $p < 0.05$, $R^2 = 0.996$) between the carbohydrate content in AH and T_{opt}, hinting that the elevated sugar

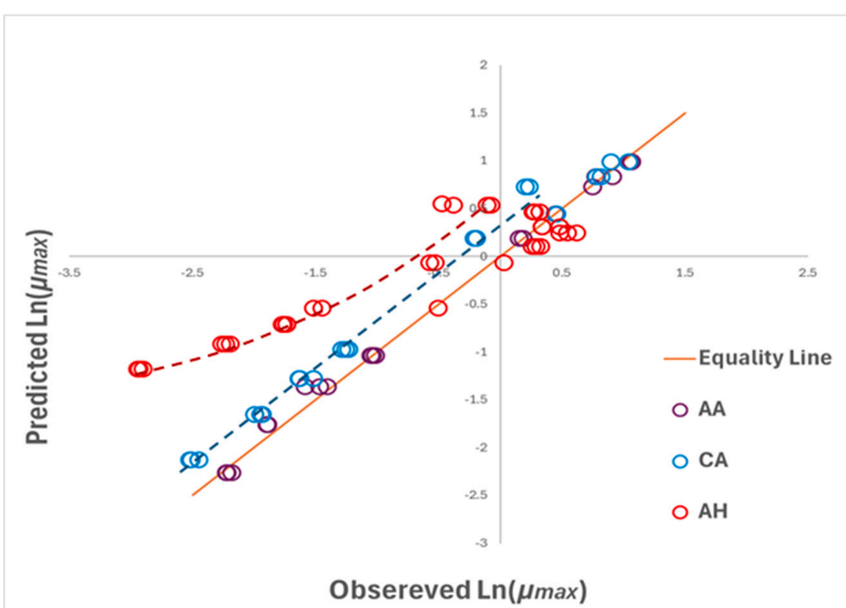

Fig. 4. Observed versus c_f-predicted natural logarithm of the maximum specific growth rates in the four studied matrices, the broken lines show the trend in these matrices in the suboptimal region.

concentrations may have been one of the reasons for the found difference. This is consistent with the observations of Santos and Martins (2003) and Yu et al. (2017) that high carbohydrate levels can exert osmotic stress, repress enzyme synthesis, or alter metabolic pathways, thereby might impact some temperature-dependent intracellular processes. Previous studies on thermophilic bacteria have reported that their maximum growth temperature (T_{max}) depends on the growth substrate and its energy yield (Heinen, 1970; Merkel and Perry, 1977; Ramaley et al., 1975). Although *Bacillus licheniformis* is typically considered thermotolerant, our observation of a significantly lower T_{opt} in a high-carbohydrate matrix supports the idea that growth temperature parameters can be modulated by matrix composition.

Ouhib et al. (2006) highlighted sugar-induced repression or stimulation of toxin and metabolite production for a closely related species, *Bacillus cereus*. Similarly, Balay et al. (2018) demonstrated that carbohydrate type and concentration significantly influence microbial growth and resistance development against antimicrobial agents, highlighting the complex interplay between matrix composition and bacterial adaptation. This further supports the need to consider carbohydrate effects when modelling microbial behavior in diverse food systems. Efforts in this direction have already begun, as shown by Nev et al. (2021), who developed growth models that incorporate variable nutrient conditions and demonstrated that key microbial growth parameters, are influenced by initial nutrient concentrations.

Fat and protein content, though less variable among the tested matrices, are also known modulators of microbial thermal kinetics (Finn et al., 2013; Geeraerd et al., 2000).

These point toward the potential value of incorporating continuous variables (e.g., macronutrient composition) instead of food classifications (e.g. almond milk alternative, coconut milk alternatives) into modelling frameworks, encouraging future research beyond the use of categorical food classifications. Incorporating such variables could bridge tertiary and secondary modelling, improving the predictive accuracy and generalizability of microbial growth models across diverse food matrices. As with the successful integration of continuous factors like pH and water activity in secondary models, this will require rigorous experimental designs to isolate the effects of individual nutrients while accounting for their interactions with environmental factors.

In summary, this study highlights the need for a more nuanced and composition-aware approach to predict bacterial growth in food, particularly as the diversity and complexity of food matrices continue to expand.

5. Conclusion and recommendation

By testing a broad range of temperatures directly in food matrices, this study provides high-resolution data for evaluating microbial growth variability under real-world conditions. Such datasets can enhance *in-silico* simulations and help quantify uncertainty in food safety modelling. The findings emphasize that, when collected transparently and reproducibly, microbial growth data should be considered, even when they depart from convenient but simplified assumptions.

For reliable use, particularly in ambient-stored products, we recommend applying the developed models only within the tested temperature range of 15 °C to 55 °C. Although the model estimates T_{min} and T_{max}, these represent extrapolations and may not reflect true biological limits.

This work draws the attention that the effect of some matrix components could be significant, beyond broad food categorizations (e.g., “almond milk”). Converting them into continuous variables would mean more advanced “tertiary modelling”.

CRediT authorship contribution statement

Maha Rockaya: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Bence Pecsenye:** Writing – original draft, Methodology, Investigation, Data curation. **Mariem Ellouze:** Writing – review & editing, Validation, Supervision, Methodology. **Endre Máthé:** Writing – review & editing, Validation, Supervision, Resources, Project administration. **József Baranyi:** Writing – review & editing, Validation, Supervision, Software, Formal analysis, Conceptualization.

Declaration of competing interest

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

Data availability

We have shared a link to our data in the Attach File step

Supplementary Material, Bacillus licheniformis growth data in BHI and three plant-based milks, Maha Rockaya (Original data) (Figshare)

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2025.111497.

References

Akkermans, S., Logist, F., Van Impe, J.F., 2018. Parameter estimations in predictive microbiology: statistically sound modelling of the microbial growth rate. Food Res. Int. 106, 1105–1113.

Aspridou, Z., Balomenos, A., Tsakanikas, P., Manolakos, E., Koutsoumanis, K.P., 2019. Heterogeneity of single cell inactivation: assessment of the individual cell time to death and implications in population behavior. Food Microbiol. 80, 85–92.

Balay, D.R., Gänzle, M.G., McMullen, L.M., 2018. The effect of carbohydrates and bacteriocins on the growth kinetics and resistance of Listeria monocytogenes. Front. Microbiol. 9, 347.

Baranyi, J., Roberts, T.A., 1994. A dynamic approach to predicting bacterial growth in food. Int. J. Food Microbiol. 23 (3–4), 277–294.

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

Baranyi, J., Buss da Silva, N., Ellouze, M., 2017. Rethinking tertiary models: relationships between growth parameters of Bacillus cereus strains. Front. Microbiol. 8, 1890.

Baranyi, J., Rockaya, M., Ellouze, M., 2024. From data to models and predictions in food microbiology. Curr. Opin. Food Sci. 57, 101177.

Buss da Silva, N., Baranyi, J., Ellouze, M., 2017. Relationships between the parameters of temperature-dependent growth models for Bacillus cereus strains. Front. Microbiol. 8, 1890.

De Jonghe, V., Coorevits, A., De Block, J., Van Coillie, E., Grijspeerdt, K., Herman, L., De Vos, P., Heyndrickx, M., 2010. Toxinogenic and spoilage potential of aerobic spore-formers isolated from raw milk. Int. J. Food Microbiol. 136 (3), 318–325.

Fan, L., Liu, S., Dai, H., Yuan, L., Yang, Z., Jiao, X.A., 2024. Genotype-phenotype evaluation of the heterogeneity in biofilm formation by diverse Bacillus licheniformis strains isolated from dairy products. Int. J. Food Microbiol. 416, 110660.

Finn, S., Condell, O., McClure, P., Amézquita, A., Fanning, S., 2013. Mechanisms of survival, responses and sources of Salmonella in low-moisture environments. Front. Microbiol. 4, 331.

Geeraerd, A.H., Herremans, C.H., Van Impe, J.F., 2000. Structural model requirements to describe microbial inactivation during a mild heat treatment. Int. J. Food Microbiol. 59 (3), 185–209.

Giugliano, R., Musolino, N., Ciccotelli, V., Ferraris, C., Savio, V., Vivaldi, B., Decastelli, L., 2023. Soy, rice and oat drinks: investigating chemical and biological safety in plant-based milk alternatives. Nutrients 15 (10), 2258.

Gopal, N., Hill, C., Ross, P.R., Beresford, T.P., Fenelon, M.A., Cotter, P.D., 2015. The prevalence and control of Bacillus and related spore-forming bacteria in the dairy industry. Front. Microbiol. 6, 1418.
Heinen, W., 1970. Growth conditions and temperature-dependent substrate specificity of two extremely thermophilic bacteria. Arch. Mikrobiol. 76 (1), 2–17.
ISO International Organization for Standardization, 2025. Microbiology of the Food Chain — Determination and Use of Cardinal Values. ISO/DIS 23691.
Kain, T., Albahri, M., Plötz, M., Jessberger, N., 2024. Growth, persistence and toxin production of pathogenic bacteria in plant-based drinking milk alternatives. J. Food Sci. 89 (9), 5799–5811.
Kyrylenko, A., Eijlander, R.T., Alliney, G., Lucas-van de Bos, E., Wells-Bennik, M.H., 2023. Levels and types of microbial contaminants in different plant-based ingredients used in dairy alternatives. Int. J. Food Microbiol. 407, 110392.
Le Marc, Y., Pin, C., Baranyi, J., 2005. Methods to determine the growth domain in a multidimensional environmental space. Int. J. Food Microbiol. 100 (1–3), 3–12.
Lücking, G., Stoeckel, M., Atamer, Z., Hinrichs, J., Ehling-Schulz, M., 2013. Characterization of aerobic spore-forming bacteria associated with industrial dairy processing environments and product spoilage. Int. J. Food Microbiol. 166 (2), 270–279.
Martinez-Rios, V., Gkogka, E., Dalgaard, P., 2019. New term to quantify the effect of temperature on pH min-values used in cardinal parameter growth models for Listeria monocytogenes. Front. Microbiol. 10, 1510.
McMeekin, T.A., Olley, J., Ratkowsky, D.A., Ross, T., 2002. Predictive microbiology: towards the interface and beyond. Int. J. Food Microbiol. 73 (2–3), 395–407.
Merkel, G.J., Perry, J.J., 1977. Effect of growth substrate on thermal death of thermophilic bacteria. Appl. Environ. Microbiol. 34 (6), 626–629.
Misiou, O., Ellouze, M., Koutsoumanis, K., 2023. Cardinal models to describe the effect of temperature and pH on the growth of Anoxybacillus flavithermus & Bacillus licheniformis. Food Microbiol. 112, 104230.
Nev, O.A., Lindsay, R.J., Jepson, A., Butt, L., Beardmore, R.E., Gudelj, I., 2021. Predicting microbial growth dynamics in response to nutrient availability. PLoS Comput. Biol. 17 (3), e1008817.
Ouhib, O., Clavel, T., Schmitt, P., 2006. The production of Bacillus cereus enterotoxins is influenced by carbohydrate and growth rate. Curr. Microbiol. 53 (3), 222–226.
Pin, C., Velasco de Diego, R., George, S., García de Fernando, G.D., Baranyi, J., 2004. Analysis and validation of a predictive model for growth and death of Aeromonas hydrophila under modified atmospheres at refrigeration temperatures. Appl. Environ. Microbiol. 70 (7), 3925–3932.
Ramaley, R.F., Bitzinger, K., Carroll, R.M., Wilson, R.B., 1975. Isolation of a new, pink, obligately thermophilic, gram-negative bacterium (K-2 isolate). Int. J. Syst. Bacteriol. 25 (4), 357–364.
Rime, J.A., 2020. Dairy Milk and Plant-based Alternative Beverage Purchasing Factors: Consumer Insights. Brigham Young University (master's thesis).
Rockaya, M., Baranyi, J., 2025. Variability of growth parameter estimates-the role of rescaling and reparametrization. Food Microbiol. 128, 104726.
Rosso, L., Lobry, J.R., Flandrois, J.P., 1993. An unexpected correlation between cardinal temperatures of microbial growth highlighted by a new model. J. Theor. Biol. 162 (4), 447–463.
Salkinoja-Salonen, M.S., Vuorio, R., Andersson, M.A., Kämpfer, P., Andersson, M.C., Honkanen-Buzalski, T., Scoging, A.C., 1999. Toxigenic strains of Bacillus licheniformis related to food poisoning. Appl. Environ. Microbiol. 65 (10), 4637–4645.
Santos, E.D.O., Martins, M.L.L., 2003. Effect of the medium composition on formation of amylase by Bacillus sp. Braz. Arch. Biol. Technol. 46, 129–134.
Sethi, S., Tyagi, S.K., Anurag, R.K., 2016. Plant-based milk alternatives: an emerging segment of functional beverages – a review. J. Food Sci. Technol. 53 (9), 3408–3423.
Setlow, P., 2006. Spores of Bacillus subtilis: their resistance to and killing by radiation, heat and chemicals. J. Appl. Microbiol. 101 (3), 514–525.
Setlow, P., Johnson, E.A., 2019. Spores and their significance. In: Doyle, M.P., Buchanan, R.L. (Eds.), Food Microbiology: Fundamentals and Frontiers, 5th ed., pp. 23–64.
Welch, B.L., 1947. The generalization of 'Student's' problem when several different population variances are involved. Biometrika 34 (1/2), 28–35.
Whiting, R.C., 1995. Microbial modeling in foods. Crit. Rev. Food Sci. Nutr. 35 (6), 467–494.
Yeak, K.Y.C., Perko, M., Staring, G., Fernandez-Ciruelos, B.M., Wells, J.M., Abee, T., Wells-Bennik, M.H., 2022. Lichenysin production by Bacillus licheniformis food isolates and toxicity to human cells. Front. Microbiol. 13, 831033.
Yu, W., Chen, Z., Ye, H., Liu, P., Li, Z., Wang, Y., He, N., 2017. Effect of glucose on poly-γ-glutamic acid metabolism in Bacillus licheniformis. Microb. Cell Factories 16, 1–10.
Zhao, J., Gao, J., Chen, F., Ren, F., Dai, R., Liu, Y., Li, X., 2014. Modeling and predicting the effect of temperature on the growth of Proteus mirabilis in chicken. J. Microbiol. Methods 99, 38–43.