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Thermal inactivation kinetics of seven genera of vegetative bacterial pathogens common to the food chain are similar after adjusting for effects of water activity, sugar content and pH

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ABSTRACT

A predictive model was made for the logarithm of the thermal decimal reduction time (logD) of *Salmonella enterica* (D = time to 90% reduction by inactivation). The model was fitted with multiple linear regression from 521 logD-values reported in literature for laboratory media and foods highly varying in water activity and pH. The single regression model with temperature as the only variable had a high residual standard error (RSE) of 0.883 logD and no predictive value (fraction of variance explained (R^2) < 0.001). Adding water activity, sugar content and pH as predictors resulted in a model with a lower RSE of 0.458 logD and an adjusted R^2 of 0.73. The model was validated by comparing 985 predicted with observed logD for *S. enterica* from other publications. The model was subsequently validated with 1498 published logD-values for inactivation of vegetative cells of nine other pathogenic bacteria genera (mainly *Listeria monocytogenes, Escherichia coli, Clostridium perfringens, Cronobacter* spp., *Staphylococcus aureus, Yersinia enterocolitica*) in or on a variety of laboratory media, meat, fish, dairy, nuts, fruits and vegetables. Regression analyses for validation with the 985 logD of *S. enterica* and 2483 logD of all genera show deviations from the expected slope of 1 (both 0.81) and the expected intercept of 0 (0.04 and 0.19 logD respectively). However, only 0.7% and 2% respectively of the new logD (expected: 0.5%) were observed above the 99% prediction interval of the original *S. enterica* model based on 521 logD. The findings suggest that i) the variability of thermal resistance of strains within species is larger than between genera and species; ii) one generic predictive model, also accounting for variability, suffices for designing the thermal inactivation of a variety of vegetative pathogenic bacteria in many food types.

Introduction

Thermal inactivation processes – e.g., pasteurization and sterilization – aim at safeguarding microbial safety and limiting spoilage of products and materials (food, pharmaceuticals, cosmetics) that may otherwise cause infections, intoxications or spoilage by micro-organisms. The designs of thermal inactivation processes are generally optimized to ensure safety and shelf life of products, while maintaining taste and nutritional value. At the same time, energy demand and other processing costs should be minimized. Inactivation kinetics of different species of bacteria and other micro-organisms are usually expressed with the variables D_{Tref}, the time to 1 log reduction of viable micro-organisms at the reference temperature (T_{ref}), and z_T, the temperature increase needed for 1 log reduction of D. Over the years, the heat inactivation of various strains in a variety of food products and laboratory media has been studied, resulting in many published D- and z-values for various conditions (e.g. ICMSF, 1996; Doyle and Mazzotta, 2000; Doyle et al., 2001, Van Asselt and Zwietering, 2006). Factors reported to have an influence on the heat resistance of a pathogen are amongst others: strain variability, growth phase (age) of the culture, growth conditions, recovery media, and characteristics of foods such as salt content, a_w, acidity, and the presence of other inhibitors (Doyle et al., 2001).

The result is a large variety of values of D_{Tref} and z_T. Quantitative microbial risk assessment models for the food industry include models

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for contamination, growth and inactivation. To account for variability of the values of model variables, these models are designed with statistical techniques, including the Monte Carlo method (Vose, 2008). These methods apply probability functions, functions fitted from published data or from observations in the particular food chain. The result of these models is a prediction interval for the probability of a concentration of a microorganism in the food chain, including survival of heat inactivation.

During the development of farm to fork chain models for the dairy industry (Van Lieverloo et al., 2007), the results of the first extensive quantitative analysis of published data concerning thermal inactivation of vegetative bacteria and bacterial spores (Van Asselt and Zwietering, 2006) were used as a source of means and standard deviations of D_{Tref} and z_T. Applying these variables in chain models for *Listeria monocytogenes* in a variety of dairy products caused unrealistically wide prediction intervals, which were to be expected, as the study was not intended for this application and only included temperature as a predictor.

Evaluating most of the available literature, including many variables of liquids (laboratory media, dairy, juices etc.) and inactivation conditions, a multiple regression model for *L. monocytogenes* was previously developed that adjusts the thermal inactivation kinetics for effects of pH, NaCl, sugar, culturing conditions and heating methods. This resulted in much narrower and more realistic prediction intervals (Van Lieverloo et al., 2013) that corroborated the observed safety of pasteurized, hygienically packaged dairy products. A basic model for *Salmonella enterica*, merely adjusting for water activity, pH and sugar content already allows for the fitting of a large variety of published D_{Tref}-values in one predictive model (Van Lieverloo and Zwietering, 2013), including even largely deviating observations in chocolate (a_w = 0.45: Goepfert and Biggie, 1968; Barille and Cone, 1970) and peanut butter (a_w = 0.33-0.7: Shachar et al., 2006; Ma et al., 2009; He et al., 2011; Keller et al., 2012). This multiple regression model had a fraction of variance explained (R^2) of 0.73 compared to 0.002 with the single regression using only temperature as descriptive variable (Van Lieverloo and Zwietering, 2013). In a subsequent comparison, the kinetics of *L. monocytogenes* and *S. enterica* proved to be very similar (Van Lieverloo et al., 2017). The contribution at hand aims to show that thermal inactivation kinetics, when adjusted for other variables, are similar for vegetative cells of a variety of common foodborne pathogenic bacteria genera and that variability of the kinetics is much higher within species.

Theory and calculation

This section summarizes the theory for multiple regression analysis; a single regression model is a linear prediction with estimated intercept $\hat{\beta}_0$ and slope $\hat{\beta}_1$ (the regression coefficient, the hat indicates 'estimated mean') in a two-dimensional plane, for thermal inactivation: $\log D_{(min)} = \hat{\beta}_0 + \hat{\beta}_1 T\,(°C) + \varepsilon$, only including T as a predictor ($\hat{z} = -1/\hat{\beta}_1$) and ε as the residual. For multiple regression analysis, the linear relation of each new predictor term (k predictors) in the model with logD is evaluated in a dimension orthogonal to the other dimensions, each with their own estimated $\hat{\beta}_k$ and $\hat{z}_k = -1/\hat{\beta}_k$. Adding pH, sugar content and a_w, but also a_w^2, T.a_w and T.a_w^2 as predictors essentially adds six new dimensions, each evaluated for a linear relation with logD. See the Methods for details on model fitting, checking and validation and the Glossary in the SI for explanation of terms.

Methods

A glossary of terms and common calculations is included in the supplementary information (SI).

Thermal inactivation model

The primary thermal inactivation model most commonly reported in literature describes the exponential reduction of the number of viable bacteria (N) as a function of time at a fixed temperature T, where D_T is the negative inverted slope of linear relation after logarithmic transformation of the viable counts: $-\partial t/\partial \log(N)$. Although other models have been successfully applied, such as the Weibull model - which often fits shoulders and tails of curves better than the linear model (Van Boekel, 2002) -, the majority of the data in literature are presented as D-values, currently limiting an analysis to these linear models.

Secondary inactivation models

A common approach to secondary models is a single linear regression fit of logD (the response) as a function of the temperature (the predictor), from which $\hat{z}_T$, the estimated mean change of temperature needed for a change of logD with 1 unit, can be calculated: the negative inverse of the estimated slope $\hat{\beta}_T$ (Eq. (1)).

$$\hat{z}_T = \frac{-1}{\hat{\beta}_T} = \frac{-\partial T}{\partial \log(D)} \tag{1}$$

Development of the multiple predictor secondary model

A multiple ordinary least squares regression model (Eq. (2)) was fitted previously to estimate the combined mean effects (regression coefficients $\hat{\beta}_p$ of each predictor variable p) on logD of changes of four predictor variables: temperature (T), pH, water activity (a_w) and sugar content (m/m) (Van Lieverloo et al., 2017).

$$\log D_{(min)} = \hat{\beta}_0 + \hat{\beta}_1 T_{(°C)} + \hat{\beta}_2 a_w + \hat{\beta}_3 a_w^2 + \hat{\beta}_4 pH + \hat{\beta}_5 Sugar_{(m/m)} + \hat{\beta}_6 T_{(°C)} a_w + \hat{\beta}_7 T_{(°C)} a_w^2 + \varepsilon \tag{2}$$

The model was developed by testing the effect of these predictors, including their second and third order polynomials and multiplicative terms, with the null hypothesis (H_0) for each term coefficient β = 0 and an alpha (acceptable probability of falsely rejecting the null hypothesis) of 0.05. The actual probabilities (*p*-values), the estimated regression coefficients ($\hat{\beta}_0$- $\hat{\beta}_7$) of the accepted variables and the residual standard error (standard deviation of ε_i) are reported in Table 1. With the sample of 521 published logD-values used for model development, a statistically significant effect of other polynomial or multiplicative terms could not be found and thus β was assumed to be 0 for these effects. Although in an alternative model (with other $\hat{\beta}$ for all other predictors, with the lowest *p*-values for H_0: β = 0) the H_0 β = 0 for fat content as an extra predictor was rejected with a *p*-value of 8.10^{-6} ($\hat{\beta}$ = 0.012 logD per 0.01 increase of fat m/m), the increase of R^2 (0.731 to 0.741) and the decrease of RSE

Table 1
Means and standard errors of the estimated coefficients ($\hat{\beta}$ and $\hat{z} = -1/(\text{mean } \hat{\beta})$, NL = not linear) for the regression model (Eq. (2)) of log(D, min) for thermal inactivation of *S. enterica* based on 521 data sets. *p* = probability of the type I error for H_0: $\hat{\beta}_{predictor} = 0$. The residual standard error is 0.4580 logD, the adjusted R^2 is 0.73.

		Estimated coefficients				*p*
Variable	Unit	$\hat{\beta}$	Mean	Standard error	Mean z	
Constant	logD	$\hat{\beta}_0$	10.23	2.93		$5.2.10^{-3}$
T	°C	$\hat{\beta}_1$	−0.1568	0.0408	[a]	$1.4.10^{-3}$
a_w		$\hat{\beta}_2$	−33.25	8.57	NL	$1.2.10^{-3}$
a_w^2		$\hat{\beta}_3$	32.02	5.98	NL	$1.3.10^{-7}$
pH		$\hat{\beta}_4$	0.1776	0.0374	−5.63	$2.6.10^{-6}$
Sugar	m/m	$\hat{\beta}_5$	1.4840	0.0905	−0.674	$< 2.10^{-16}$
T . a_w		$\hat{\beta}_6$	0.5599	0.1254	NL	$9.8.10^{-6}$
T . a_w^2		$\hat{\beta}_7$	−0.5754	0.0897	NL	$3.3.10^{-10}$

[a] $\hat{z}_T$ is dependant on water activity. At $a_w = 1$, the fitted $\hat{z}_T = -1/(\hat{\beta}_1+\hat{\beta}_6+\hat{\beta}_7) = -1/-0.1723 = 5.80$.

(0.458 to 0.450) was not considered enough. As the data also showed heteroscedasticity (high variance at a fat content near 0 m/m) and considering the objective of parsimony, the model without fat content was selected.

Partial regression scatter plots of the fitted model were used to visually check the requirement of homoscedasticity for each predictor. These plots were constructed by using all model coefficients from Table 1, fixing the values of all predictors but one, using reported values of the remaining predictor and adding the model residuals (ε). For example, the partial regression model for T was constructed using Eq. (2) to predict logD for the actual T of each observation (numbered i) from the literature and a fixed a_w (0.993), pH (6.5) and sugar content (0.046 m/m) to values of whole milk, resulting in Eq. (3). The estimates of the preliminary model residuals ($\hat{\varepsilon}$) are added to allow for visual evaluation of homoscedasticity, leverage and decrease of residual standard error. An alternative notation of Eq. (3) is given in Eq. (4).

$$\log D_{(i,\min)} = \hat{\beta}_0 + \hat{\beta}_1 T_{(i,^\circ C)} + \hat{\beta}_2 0.993 + \hat{\beta}_3 0.993^2 + \hat{\beta}_4 6.5 + \hat{\beta}_5 0.046 + \hat{\beta}_6 T_{(i,^\circ C)} 0.993 + \hat{\beta}_7 T_{(i,^\circ C)} 0.993^2 + \varepsilon_i \quad (3)$$

$$\log D_{(i,\min)} = \left(\hat{\beta}_0 + \hat{\beta}_2 0.993 + \hat{\beta}_3 0.993^2 + \hat{\beta}_4 6.5 + \hat{\beta}_5 0.046\right) + \left(\hat{\beta}_1 + \hat{\beta}_6 0.993 + \hat{\beta}_7 0.993^2\right) T_{(i,^\circ C)} + \varepsilon_i \quad (4)$$

Model validation

The model was validated by predicting logD with the inactivation model (Eq. (2)) using the temperature, water activity, pH and sugar content as reported in other publications (Table SI-1) and comparing the logD-values reported in these publications (called 'observed') with these predictions (called 'predicted'). Scatter plots of observed vs. predicted logD are presented in graphs, including the line of the expected mean (observed = predicted) and the 99% prediction interval with a margin of 1.184 logD (at 513 degrees of freedom: d.f.). Considering the main purpose of this evaluation and the high number of observations, the margin is presented as constant (for calculation and details, see Glossary in the SI). The mean regression line of observed vs. predicted logD is included in the scatter plot as an indicator of accuracy (intercept $\hat{\beta}_0$ and slope $\hat{\beta}_1$) and precision (residual standard error: RSE). The mean prediction error (observed – predicted) was calculated, as another measure of accuracy, as well its standard deviation (SD), as a measure of precision not adjusted for β_0 and β_1 of the regression.

Data sources for development of the predictive model

For each experiment reported in literature, a data set consisting of the D-value at the experimental temperature T was supplemented with data regarding the composition of the heating medium or food product and experimental conditions (culturing, conditioning, isolation, storage, inoculation, heating/cooling, enumeration). In a preliminary analysis of this database the results of 521 experiments from literature were used to fit a basic model for inactivation of *S. enterica* (Van Lieverloo et al. 2017), and this secondary model included T, pH, a_w and sugar content as predictors (Eq. (2)). The data were extracted from 19 articles on thermal inactivation of *S. enterica* from 1968 to 2012, reporting 21 strains of serotypes Typhimurium (147 data sets), Senftenberg (91), Bedford (48), Enteritidis (34), Anatum (33), Tennessee (25), cocktails (69) and others (82). Condition ranges were: T 48–90 °C; pH 5.1–9.3; a_w 0.33–0.998 ($\leq$ 0.5 in chocolate or peanut butter), sugar content (0–0.78 m/m), sodium chloride content (0–0.201 m/m) and fat content (0–0.5 m/m). Foods and media reported were: chocolate (22 data sets), liquid egg (54), peanut butter (49), water (61), heart infusion broth (134), McIlvaine citrate phosphate buffer (18), nutrient broth (15), and phosphate buffer (168). Observations from experiments with antimicrobial agents, heat shock or prior adaptation to extreme pH or a_w conditions were excluded as inclusion leads to a more complex model and a more unbalanced data set, often leading to heteroscedasticity. Details and references of the data sources are included in Table SI-1 in the SI.

Data sources for validation of the predictive model

The model was validated for predicting inactivation of *S. enterica* using data from other publications. The model was also tested for its validity to predict inactivation of other genera. D-values from 2483 experiments were collected from 118 articles published between 1968 and 2013 on thermal inactivation (laboratory media: 932; meat: 618; dairy: 505; egg 263; nuts: 113, fruits: 68; fish: 18; vegetables: 18). These included 985 new data sets for *S. enterica* (30 strains + cocktails heated in or on media, egg, meat, fish, fruits, nuts, dairy), vegetative cells of *Clostridium perfringens* (129, meat and media), *Cronobacter* spp. (originally named: *Enterobacter sakazakii*, 79 data sets from dairy and media), *E. coli* (259, meat, media, dairy, fruit), *Listeria monocytogenes* (801, media, dairy, egg, meat, fruit, vegetables), *Staphylococcus aureus* (81, media, dairy, vegetables), *Y. enterocolitica* (61, dairy, meat) and eight other species (88 data sets from four genera (in dairy, meat, media) see Table 1 and SI1). Ranges of the conditions in the experiments reported in the references providing validation data were: T 47 - 90 (°C); a_w 0.2 - 1; pH 3 - 10; sugar 0 - 0.7 (m/m); sodium chloride 0–0.201 (m/m) and fat 0–1.0 (m/m). Observations in foods with artificially adapted a_w or at 126 °C were excluded from data retrieved from He et al. (2013; peanut butter with added moisture) and Phungamngoen et al. (2011; dried cabbage). Details of foods and media, experimental condition ranges, and references of the data sources are included in Table SI-1 tab A in the SI.

Supplementary sources of composition of food products and laboratory media

Description of the conditions in food products and media used in many articles are limited, often even lacking information on water activity, pH or contents. Information on composition of foods and commonly used commercially supplied laboratory media is not available decades later and can only be assumed to be similar to foods and media currently available on websites of the same or other suppliers. Missing data were complemented with data from similar (mostly generic) foods, mostly from the USDA food composition database (FoodData Central, 2021). A list of these sources is provided in Table SI-1 (sheet B: per paper and sheet C: data from one or more papers used as standard value).

Water activity

In many publications, the water activity of the heating medium or food product was not reported. Most of the missing values were supplemented with data from other papers, using similar products. A list of these sources is provided in Table SI-1 tab C.

A calculation tool (Rouweler, 2013) was used to estimate the remaining missing 322 water activities (192 data sets (DS) $\geq$ 0.99; 0.99 > 67 DS $\geq$ 0.98; 0.98 > 38 DS $\geq$ 0.95; 0.95 > 22 DS $\geq$ 0.73; 3 DS at a_w = 0.61) from the composition of the laboratory media (230 DS) and beef

and chicken gravy (92 DS). The tool is freely available from the world wide web and is added to the SI for the sake of traceability. The parts of the formula used from the tool, in this paper applied for NaCl 0.05% - 9% (278 DS), sugars (glucose ≤ 9% (156), fructose 30% and 70% (4), sucrose 26%−70% (24) and lactose 0,3% (45)) and polyols (glycerol 30%−70% (6) and sorbitol 30%−70% (4)) is included in Table SI-1 sheets C and D.

pH

For most D-values, the pH of the heating medium or food product was provided in the original publication, related publications (usually the same research group), books or from websites of suppliers of laboratory media (although recipes possibly changed since publication). A list of these sources is provided in Table SI-1 sheets B and C.

Variability between and with genera/species

To evaluate similarity of thermal inactivation kinetics, variability between the seven species of seven genera with enough observations ($N > 60$, Table 2) and within these species were compared, using two methods:

1 Comparing the mean prediction errors (observed – predicted; (Table 3)
2 Comparing the intercepts $\beta 0_{,species}$ of each species in a combined validation regression (observed vs. predicted; Table SI-2).

Statistical and graphical software

All statistical analyses were performed using R version 3.6.2 (R Core Team, 2019) with the RStudio interface version 1.2.5033 (RStudio, 2019). The SI provides details on the functions used in R:

- 'stats' package: 'model <- lm(formula)'; 'plot (model)'; 'aov'; 'TukeyHSD'.
- 'car' package: 'vif(model)'

Graphs were made with Microsoft Excel ® Office 365 ProPlus.

Results

Preliminary secondary inactivation model for Salmonella enterica

Fig. 1A shows the single regression scatter plot of 521 logD vs T with $p(H_0: \beta_T = 0) = 0.24$, a residual standard error (RSE) of 0.883 logD and $R^2 < 0.001$ for *S. enterica*, indicating there is essentially no mean effect of T on logD, due to relevant effects of other relevant variables. By adding a_w as a linear predictor, the *p*-values of the regression coefficients for both temperature as well as a_w are lowered to $< 1.10^{-16}$ and the adjusted R^2 for this model is 0.46. This clearly shows that it is essential to adjust for the effect of differences in a_w, as the experiments include low a_w foods such as peanut butter (a_w 0.33 - 0.7), chocolate (0.45), chocolate syrup (0.75–0.83), liquid egg (0.75–0.998) and media (0.7–0.998). Adding a_w^2, pH, sugar and the multiplicative effects of $T.a_w$ and $T.a_w^2$ further improves the model (Eq. (2) in Methods). Table 1 shows the coefficients of all predictor variables (mean $\widehat{\beta}$, standard error and $\widehat{z} = -1/\widehat{\beta}$ for independent linear effects) of this predictive inactivation model for *S. enterica*. The probabilities p of the type I error (falsely rejecting H_0: $\beta = 0$) for each coefficient are 0.0012 or (much) lower, the RSE is 0.458 logD and the adjusted R^2 is 0.73 (Table 1). Model checking with R-functions 'plot(model)' and 'vif(model)' resulted in acceptable deviations from modelling assumptions concerning residuals (homoscedasticity, normal distribution, Cook's distance (measure for leverage) < 1 and variation inflation factors, see Methods and Glossary). The partial regression plots for T (Fig. 1B) at $a_w = 0.993$ and $a_w = 0.400$ show a homoscedastic partial model for T and the effect of a_w on T: z_T decreases from 40 °C at $a_w = 0.4$ to 6 °C at $a_w = 0.993$. Similar single regression and partial regression plots at $T = 55$ °C and $T = 72$ °C for a_w (Fig. 2), for pH (Fig. 3) and sugar content (Fig. 4) show the effect of adding predictors other than T as decrease of variance of the residuals and an improvement in homoscedasticity (constant variance). The mean model coefficients in Table 1, visualized in the partial regression plots, indicate that:

- LogD is decreasing linearly with increasing temperature, but less rapidly at lower a_w (Fig. 1).
- Lowering the a_w increases the thermal resistance, but less so at lower temperatures, and there seems to be a limit to the lowering effect of

Table 2
Results of validation of the thermal inactivation model (Eq. (2) and Table 1) based on 521 *S. enterica* data sets. *N* = number of observations; PI99 = 99% prediction interval; *P* = proportion of all observations (%), above (expected: 0.5%) and outside (expected: 1%); Mean = mean of prediction errors (observed minus prediction); β_0 and β_1 are the intercept (expected 0) and the slope (expected 1) respectively of the model validation regression (observed vs. predicted logD). Measures of precision are SD (standard deviation) of the prediction errors and RSE (residual standard error) of the validation regression. For comparison, RSE's are included of the multiple regression model fit (0.46) and the single regressions (RSE '06) of logD vs. T, each with their own β_0 and β_T, from Van Asselt & Zwietering (2006). See Methods and Glossary (in the SI) for differences in calculations.

Species	Observed	Above PI99		Outside PI99		Accuracy			Precision			Figure no.
	N	*N*	*P*	*N*	*P*	Mean	β_0 [a]	β_1 [a]	SD	RSE	RSE '06	
Model fit												
S. enterica	521	1	0.2%	5	1.0%					0.46		1-4
Model validation												
All species	2483	49	2.0%	87	3.5%	0.20	0.19^{-63}	0.81^{-47}	0.59	0.54		
Species with $N > 60$	2395	48	2.0%	61	2.8%	0.22	0.22^{-83}	0.86^{-27}	0.58	0.52		
											Fits '06	
S. enterica (new data)	985	7	0.7%	21	2.1%	0.05	$0.04^{-1.3}$	0.81^{-17}	0.59	0.56	0.72 [b]	5
C. perfringens	129	7	5.4%	8	6.2%	0.61	0.69^{-36}	0.32^{-8}	0.77	0.41	0.37	8
Cronobacter spp.	79	0	0%	0	0%	0.09	$0.10^{-1.0}$	0.96^{0}	0.47	0.46	0.47	10
E. coli	259	17	6.6%	19	7.3%	0.50	0.49^{-49}	$0.93^{-1.3}$	0.65	0.42	0.62	7
L. monocytogenes (LM)	801	7	0.9%	8	1.0%	0.30	0.29^{-81}	$0.96^{-1.8}$	0.48	0.37	0.40 [b]	6
S. aureus	81	10	12.3%	11	13.6%	0.56	0.46^{-10}	0.59^{-11}	0.92	0.54	0.47	9
Y. enterocolitica	61	0	0%	2	3.3%	−0.10	-0.10^{0}	1.01^{0}	0.45	0.44	0.44	11
Campylobacter spp.	35	0	0%	5	14.3%	−0.71	$-0.35^{-1.6}$	0.66^{-2}	0.83	0.41	0.50	SI-1
Listeria spp. (not LM)	24	0	0%	0	0%	0.19	0.18^{-7}	0.93^{-2}	0.24	0.10		SI-2
S. pyogenes	9	1	11.1%	1	11.1%	−0.01	$-0.58^{-1.7}$	0.52^{-2}	0.63	0.39	0.57	SI-3
Vibrio spp.	20	0	0%	15	75.0%	−1.4	-0.19^{0}	0.23^{-9}	1.64	0.22	0.46	SI-4

[a] log(p) of significance levels for rejecting H_0 ($\beta_0 = 0$) and H_0 ($\beta_1 = 1$) are indicated as superscripts of β_0. $\beta_0{}^{x}$ indicates β_0 with $p(\beta_0 = 0) < 10^{x}$. $\beta_0{}^{0}$ indicates $p > 0.1$.
[b] Without experiments in low a_w food products: chocolate (*S. enterica*) and salted products (*L. monocytogenes*).

Table 3
Mean prediction errors (observed minus predicted) of logD of seven species (2395 data sets) and the results of analysis of variance post hoc pairwise testing of differences (d) between these means using Tukey-adjusted probabilities p of falsely rejecting H_0 ($d = 0$). < indicates that $p < 1.10^{-6}$. The last two columns show the standard deviations of the prediction errors per species (SD_{errors}) and the result of the Levene's test for comparing these standard deviations with the standard deviation of the means of the seven species (0.28 logD), where p is the probability p of falsely rejecting H_0 (SD of seven means of errors = SD of errors of one species). A similar table, comparing intercepts of a validation regression model for all seven species, is included as Table 3.

Species	n	Mean error (logD)	(/RSE)[a]	Tukey-adjusted p (pairwise comparison) SE	CP	CS	EC	LM	SA	SD_{errors} (logD)	Levene's test (p)
S. enterica new data (SE)	985	0.05	11%							0.58	0.09
C. perfringens (CP)	129	0.61	133%	<						0.46	0.40
Cronobacter spp. (CS)	79	0.09	20%	0.99	<					0.46	0.15
E. coli (EC)	259	0.50	109%	<	0.35	<				0.42	0.48
L. monocytogenes (LM)	801	0.30	66%	<	<	0.008	<			0.37	0.56
S. aureus (SA)	81	0.56	122%	<	0.99	<	0.97	2.10^{-4}		0.73	0.20
Y. enterocolitica	61	−0.10	−22%	0.28	<	0.29	<	<	<	0.44	0.52

[a] As proportion of the RSE of the predictive model (0.46 logD) in Eq. (2) with coefficients from Table 1.

a_w (Fig. 2). This multiplicative effect of temperature and a_w corroborates results of earlier studies e.g. by Jin et al. (2020).

- LogD increases with pH, although a limited number of observations at pH above 7.5 suggest that the effect of pH may follow an optimum curve (Fig. 3). The limited number of observations above pH=7.5 do not supply enough information to fit a polynomial of pH describing such an optimum curve.
- Increasing the sugar content further increases logD, suggesting that lowering a_w by adding sugar, known to protect vegetative cells (Gibson, 1973; Corry, 1974; Sumner et al., 1991), is more effective in increasing resistance to thermal inactivation than lowering a_w with other means (low moisture or adding other components affecting a_w, such as NaCl and other salts). In the literature data used, experiments in heating media or food products with sugar contents above 0.7 m/m (all from Gibson, 1973) show logDs lower than the mean fit, suggesting the effect of sugar content may follow an optimum curve (Fig. 4).

Validation with other S. enterica data

Inactivation logDs were predicted using the model for *S. enterica* (Eq. (2) with mean coefficients from Table 1) and values of T, a_w, pH and sugar content from 985 data sets on this species retrieved from other publications. The results of the validation, comparing observed logDs (reported) and predicted logDs, are presented in Table 2 and in Fig. 5.

The mean of the prediction errors (0.05 logD) and the intercept ($\beta_0 =$ 0.04 logD) of the regression of observed vs. predicted logD are related measures of systematical error as part of the evaluation of accuracy. As p ($\beta_1 = 0$) = 0.045, a slight constant underestimation of logD is likely.

The slope ($\beta_1 = 0.81$) of the regression model of observed vs. predicted logD is another measure of accuracy: the deviation from the expected slope of 1 is highly significant: $p(\beta_1 = 1) < 10^{-17}$, indicating underestimation of low logD (short heating, usually at higher temperatures) and overestimation of high logD (long heating, usually at lower temperatures).

A higher observed than predicted logD indicates a fail-dangerous underestimation of logD required. The proportion of predictions above the 99% prediction interval (PI99) has an expected value of 0.5%. Seven of the 985 observed logDs (0.71%) were above the PI99, one of which at a low predicted logD.

The standard deviation (SD) of the observed – predicted logD and the residual standard error (RSE) of the validation regression of observed vs. predicted logD are measures of precision of the predictive model (for calculations, see the Glossary in the SI). The SD is the precision of observations relative to the line indicating identical values of observed and predicted logD in Fig. 5. The RSE is the precision relative to the regression line of observed vs. predicted logD in this figure and is adjusted for the effect of systematical errors quantifies as the validation regression intercept β_0 and slope β_1. These measures can be compared to the measures of precision of the predictive regression model. The RSE of the validation regression is 0.59 logD, which is higher than the expected 0.458 logD (the RSE of the predictive model).

Validation of applicability for vegetative cells of other bacteria genera

The model was then also tested to see how well it could predict the inactivation of vegetative cells of the nine other genera. The results of the validation, comparing observed logD (reported in literature) and predicted logD as explained for the validation with new data of *S. enterica*, are presented in Table 2 for the combined data and per species. The validation results are presented in graphs for the species for which the more than 60 observations were found: *L. monocytogenes* (Fig. 6), *E. coli* (Fig. 7), *C. perfringens* (Fig. 8), *Staphylococcus aureus* (Fig. 9), *Cronobacter* spp. (Fig. 10) and *Y. enterocolitica* (Fig. 11). Figures for the other genera/species are included in the SI.

The mean prediction error and the intercept β_0 show positive numbers for most species, indicating an underestimation of logD. The values overall are near 0.20 for all data combined and range from −0.10 (*Y. enterocolitica*) to 0.49 (*E. coli*) and 0.69 (*C. perfringens*) for species with more than 60 observations (Table 2). These systematic errors are not large in comparison to the RSE of the predictive model (0.458 logD) and the resulting 99% prediction interval (PI99) of 2.368 logD, but statistically significant from 0 for all species except for *Cronobacter* spp. ($p = 0.09$) and *Y. enterocolitica* ($p = 0.11$).

The validation regression slope β_1 is 0.81 for all species combined (2483 observations, Table 2), similar to that of *S. enterica* alone (Fig. 5, 895 observations). Limiting the validation to the data from the first seven species in Table 2 (61 to 895 observations per species) results in a slope of 0.86. The slopes for most species statistically significantly differ from the expected 1, but differences are small for *Listeria monocytogenes* (0.96; $p = 0.02$; Fig. 6), *E. coli* (0.93; $p = 0.05$; Fig. 7), *Cronobacter* spp. (0.96; $p = 0.66$; Fig. 10), and *Y. enterocolitica* (1.01; $p = 0.88$; Fig. 11). For *C. perfringens* (Fig. 8), the slope is 0.32 (Table 2), partly due to leverage from outliers, possibly caused by spore formation (100 of 127 observations were in heated meat products). Outliers have less effect on the slope of the predictions for *S. aureus* (Fig. 9), which is 0.59 (Table 2).

The proportion of logD above the PI99 is highest for *C. perfringens* (5.4%), *E. coli* (6.6%) and *S. aureus* (12.3%) and is 2% for all species combined (Table 2). Lifting the whole PI99 by increasing the intercept of the predictive model with 0.20 logD would decrease the proportions of observations above the PI99 to 0.8% for all species, to 1.6% for *C. perfringens*, to 0.8% for *E. coli*, to 0.1% for *L. monocytogenes* and to 0.5% for *S. enterica*. This is not the case for *S. aureus*, for which still ten out of 81 (12.3%) of observed logD would be above the prediction interval. Of these ten data sets, eight are from Kornacki & Marth (1989) who found very high D-values at 58 °C and at 77–79 °C in cow milk.

The SDs of the prediction errors are highest for *S. aureus* (0.92 logD), *C. perfringens* (0.77) and *E. coli* (0.65) and these SDs include the effects of

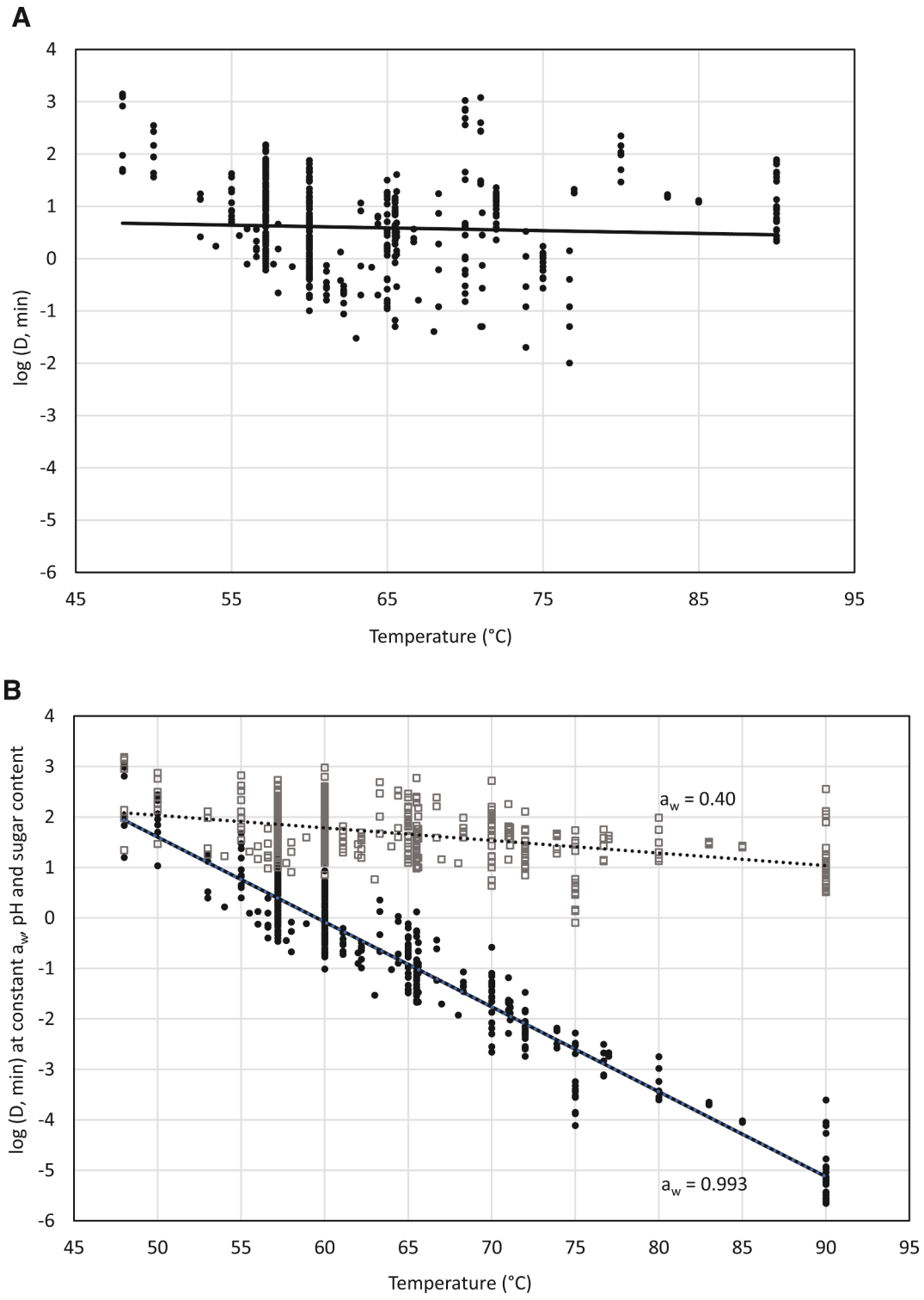

Fig. 1. Effect of temperature in the thermal inactivation model fitted from 521 literature data sets for *S. enterica*. Graph A: Single regression of logD vs. T; Graph B: partial regression for T of the fitted multiple regression model, using fixed values of a_w (0.400: open squares and 0.993: closed circles), pH (6.5) and sugar content (0.046 m/m).

the accuracy errors (mean, β_0 and β_1) described above (Table 2). The RSEs of the validation regressions (Table 2) are all close to the RSE of the predictive model (0.458) and are close to the RSEs of the single regression models presented by Van Asselt & Zwietering (2006) in their analysis, in which they allow the slope (and therefore z) to vary and exclude data with low a_w.

Differences within a species and between species or genera

The mean prediction errors (observed – predicted logD), part of accuracy evaluation in Table 2, were compared pairwise (with Tukey HSD), after analysis of variance of the residuals. The differences are small - although most are statistically highly significant - when expressed as

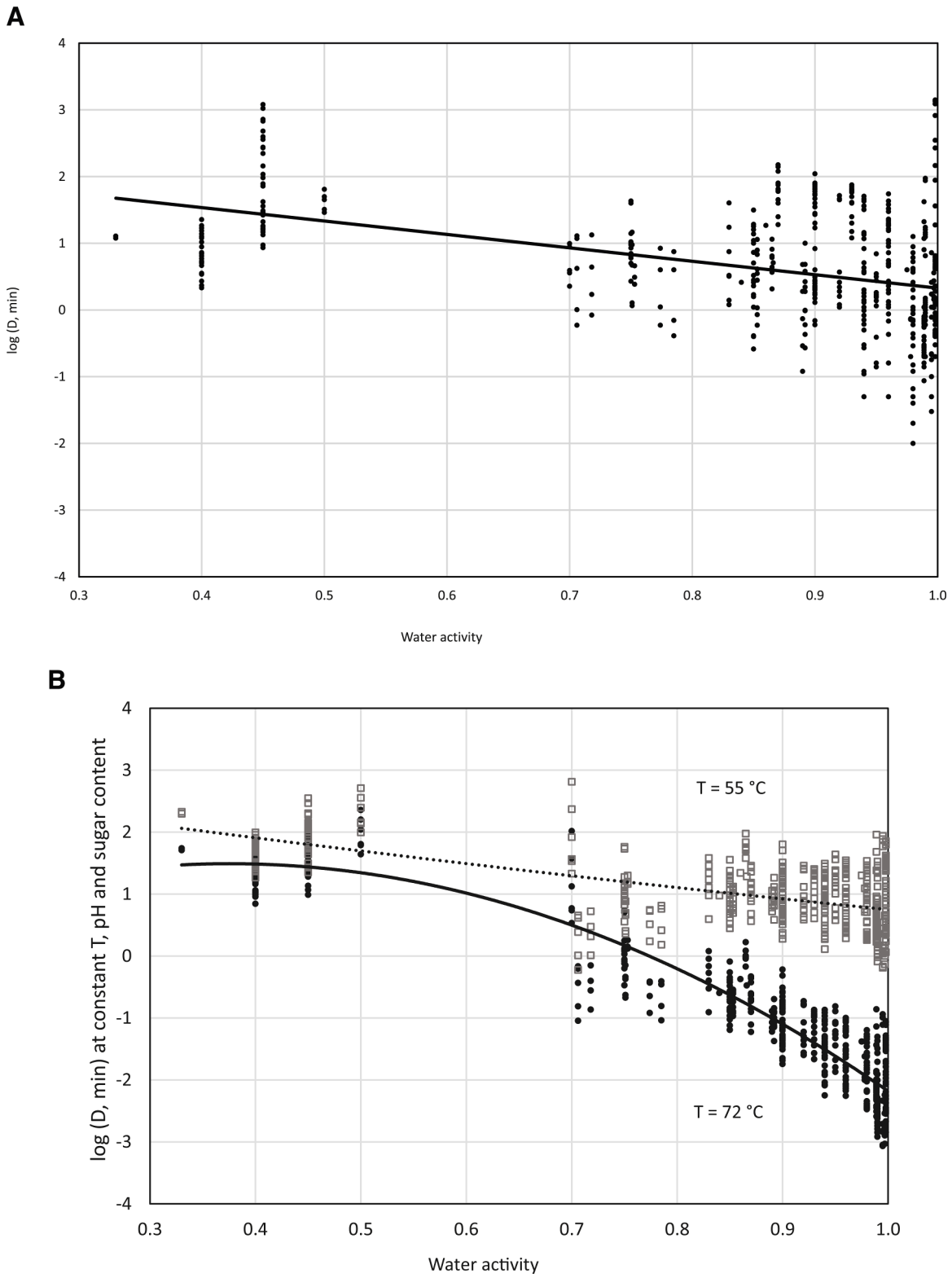

Fig. 2. Effect of water activity in the thermal inactivation model fitted from 521 literature data sets for *S. enterica*. Graph A: single regression plot logD vs. a_w. Graph B: Partial regression for a_w at fixed values of T (55 °C: open squares and 72 °C: closed circles), pH (6.5) and sugar content (0.046 m/m).

proportions of the RSE of the preliminary prediction model of 0.4580 logD (11% - 133%, Table 3) and 5.2 times lower (2% - 26%) compared to the width of the 99% prediction interval (PI99 = 2.368 logD, Figs. 5 through 11). The standard deviations for each species vary from 0.38 to 0.72 logD, higher than the standard deviation of the seven intercepts (0.26 logD), albeit not statistically significantly so (Table 3, Levene's $p \geq 0.05$).

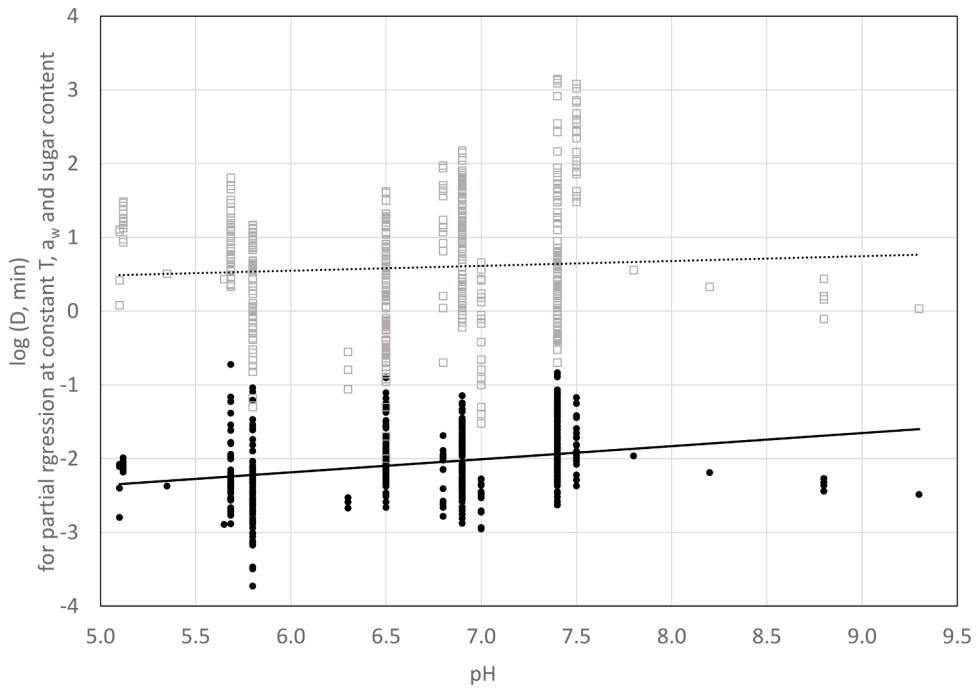

Fig. 3. Effect of pH in the thermal inactivation model fitted from 521 literature data sets for *S. enterica*. Open squares, dotted line: single regression plot logD vs. pH. Closed circles, solid line: Partial regression for pH at fixed T (72 °C), a_w (0.993) and sugar content (0.046 m/m).

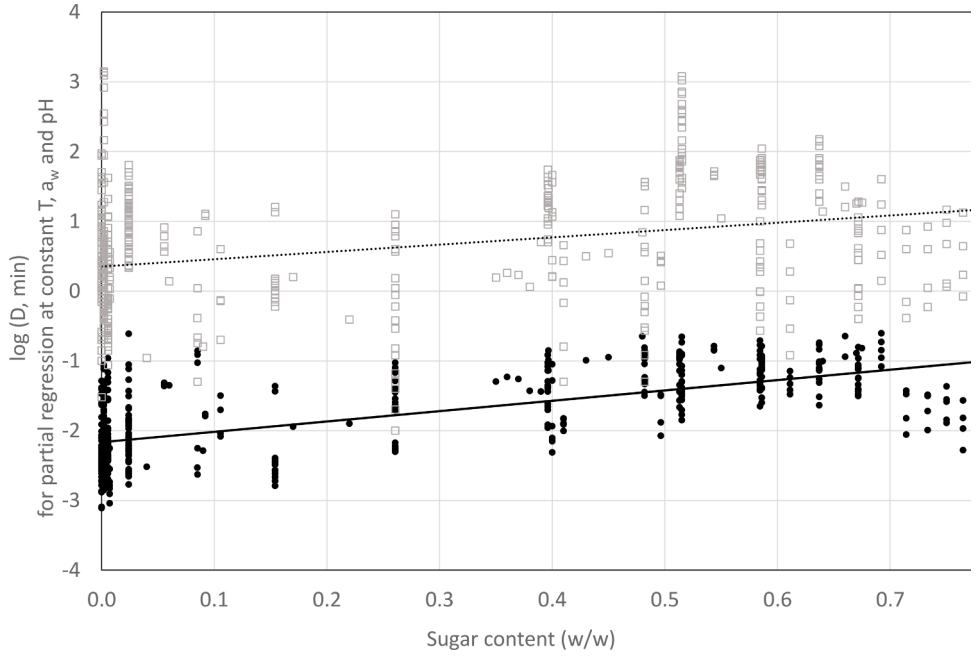

Fig. 4. Effect of sugar in the thermal inactivation model fitted from 521 literature data sets for *S. enterica*. Open squares, dotted line: single regression plot logD vs. sugar. Closed circles, solid line: Partial regression for sugar at fixed T (72 °C), a_w (0.993) and pH (6.5).

A similar analysis, comparing intercepts β_0 of validation regressions (observed vs. predicted logD) shows almost identical results (Table SI-2 in the SI).

These results show that the variability between common pathogenic species of seven bacteria genera is smaller than or at least similar to the variability within species.

General discussion

Limitations of analyses of literature data

The data set is constructed from a large number of (mostly) independent experimental data sets, obviously not forming a full factorial or balanced fractional factorial randomized block design. The effects of many combinations of the four predictors for inactivation, namely, T, a_w, pH and sugar content have not been published for all species and food or media types presented here. The results therefore should not be

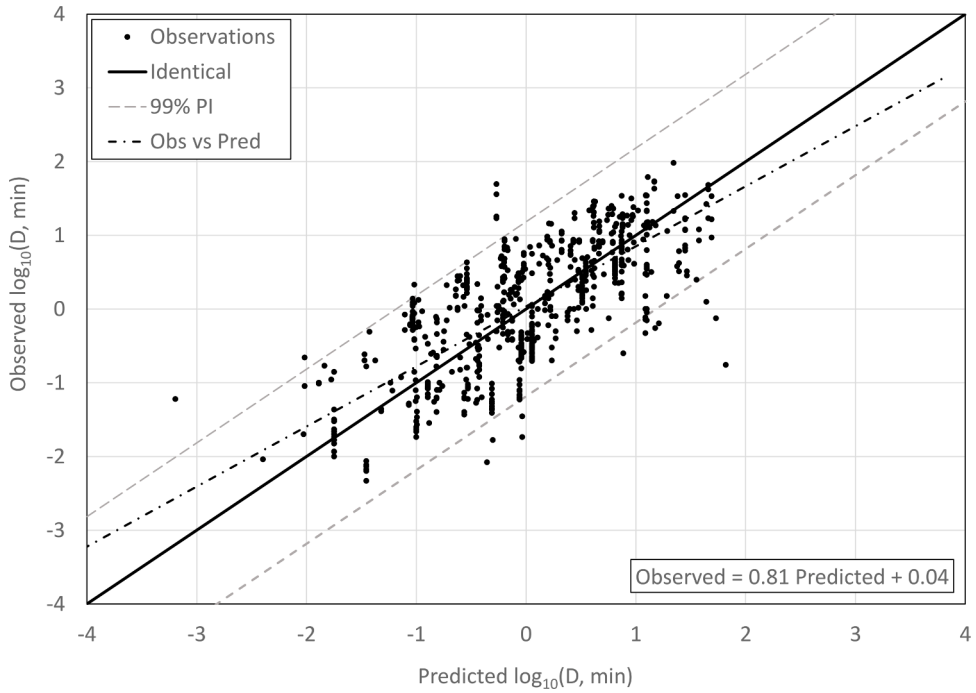

Fig. 5. Observed vs. predicted logD of inactivation of *S. enterica*. Predictions were made with the model for inactivation of *S. enterica* (fitted from 521 data sets) using T, a_w, pH and sugar content of 985 new data sets providing logD. 99% PI = 99% prediction interval for a single prediction. The formula shows the slope and intercept of the regression line of observed vs. predicted logD (Obs vs Pred).

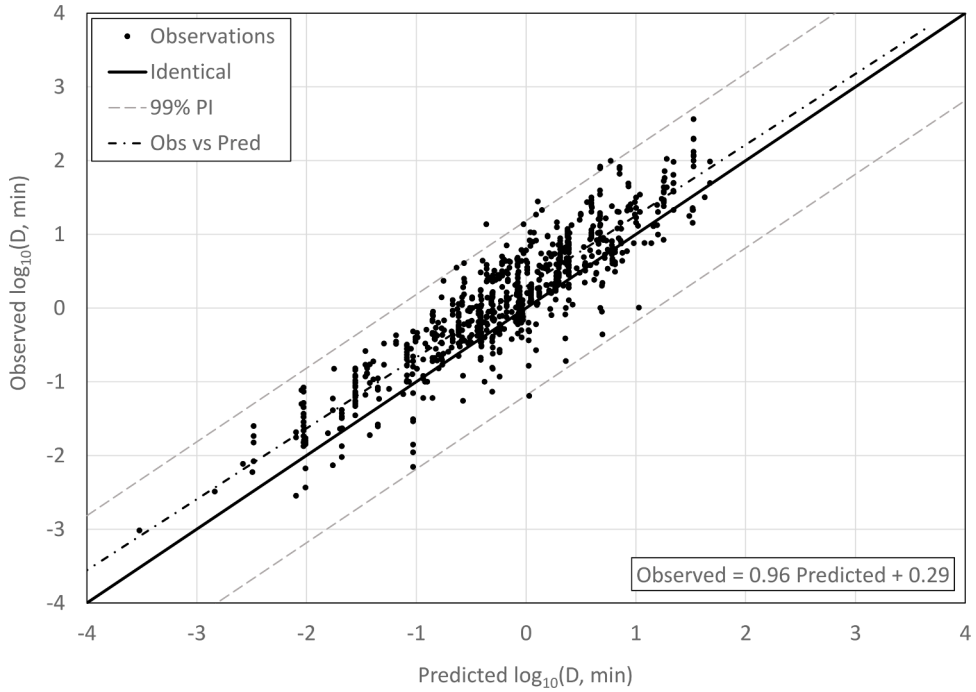

Fig. 6. Observed vs. predicted logD of inactivation of *Listeria monocytogenes* (LM, 801 data sets). Predictions were made with the model for inactivation of *S. enterica* using T, $a_{w,}$ pH and sugar content from the LM data. 99% PI = 99% prediction interval for a single prediction. The formula shows the slope and intercept of the regression line of observed vs. predicted logD (Obs vs Pred).

considered the formal result of experimental hypothesis testing. It can only be used to form hypotheses for experiments that do follow a formal design. Using all of the data presented here for validating the *S. enterica* model, to fit an overall model for all species, would further decrease the balance of design and introduce more heteroscedasticity in the regression model.

For linear regression, formally, all observations of the predictor variable should be independent. This is not true for all predictor variables, as the values of aw, pH and sugar contents are assumed identical, based on one or few measurements or even assumptions, while the value should formally be determined for each individual D-value observed.

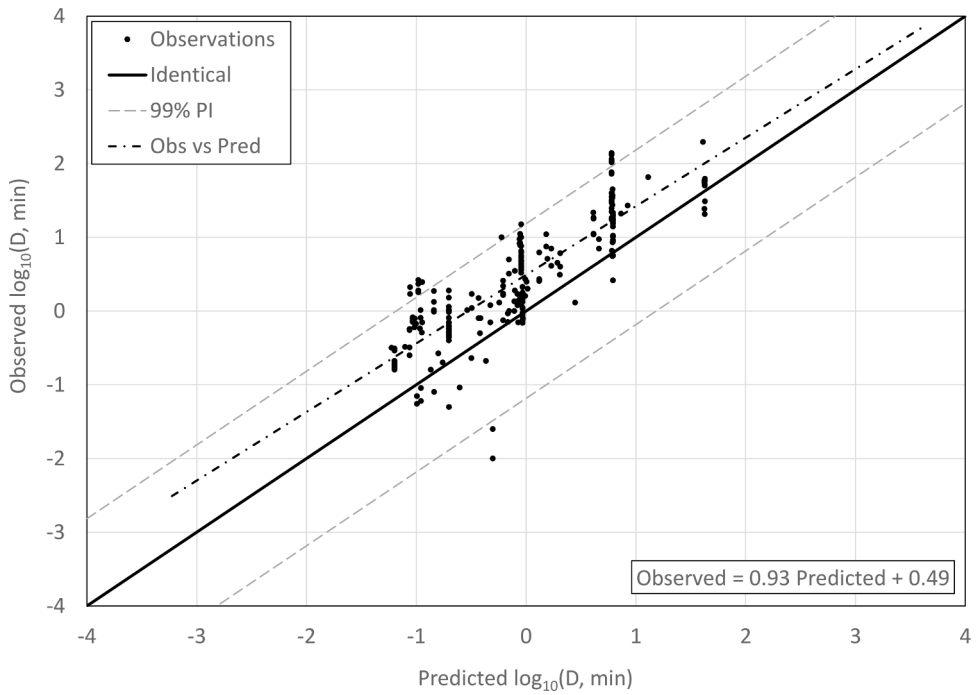

Fig. 7. Observed vs. predicted logD of inactivation of *Escherichia coli* (EC, 259 data sets). Predictions were made with the model for inactivation of *S. enterica* using T, a_w, pH and sugar content from the EC data. 99% PI = 99% prediction interval for a single prediction. The formula shows the slope and intercept of the regression line of observed vs. predicted logD (Obs vs Pred).

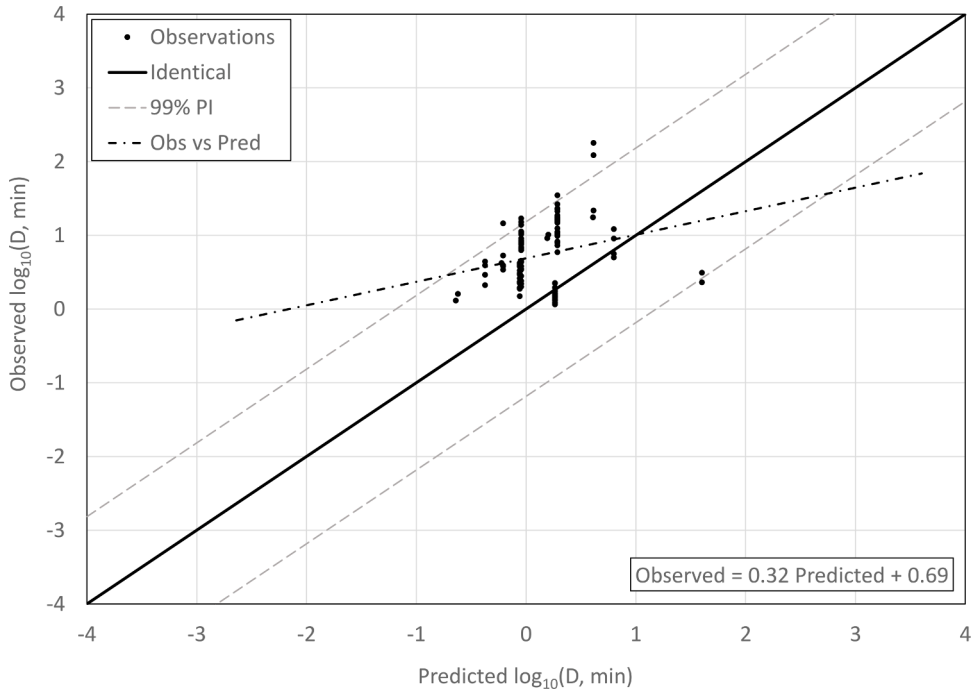

Fig. 8. Observed vs. predicted logD of inactivation of vegetative cells of *Clostridium perfringens* (CP, 129 data sets, in meat and media). Predictions were made with the model for inactivation of *S. enterica* using T, a_w, pH and sugar content from the CP data. 99% PI = 99% prediction interval for a single prediction. The formula shows the slope and intercept of the regression line of observed vs. predicted logD (Obs vs Pred).

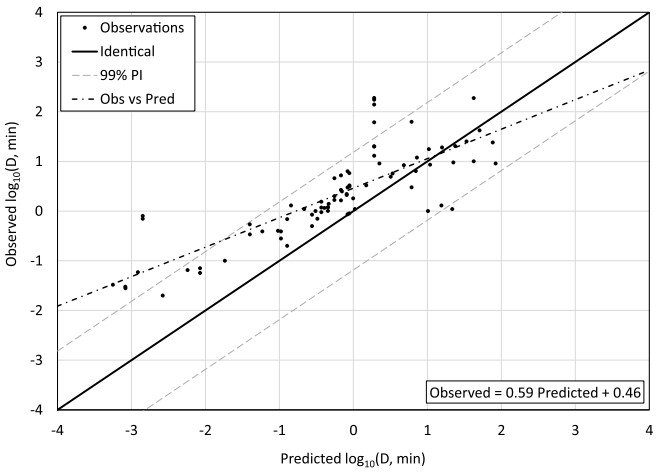

Fig. 9. Observed vs. predicted logD of inactivation of *Staphylococcus aureus* (SA, 81 data sets). Predictions were made with the model for inactivation of *S. enterica* using T, a_w, pH and sugar content from the SA data. 99% PI = 99% prediction interval for a single prediction. The formula shows the slope and intercept of the regression line of observed vs. predicted logD (Obs vs Pred).

Causes of residual variability

The residual variability within species most likely is caused by:

- Inaccuracy of assumptions regarding a_w, pH and sugar contents not provided in the original articles. The process of estimation used is likely to decrease to the predictive value of the model.
- Variability in composition – besides a_w, pH and sugar - of heating media or food products and the culturing conditions of the bacteria before and after thermal inactivation. Adjusting for these effects on logD as well as on the relations between logD and the other predictors (e.g. carbohydrates and other macronutrients affecting water activity at high temperatures (Jin et al., 2019)), would result in a narrower prediction interval.
- Complex foods are sometimes not homogeneous and may have different microvariations in water activity values.
- Variability between experiments, especially due to differences in selected heating conditions and methods.
- Variability between experiments performed under identical conditions (reproduction variability).
- Variability within experiments (experimental variability).
- Strain variability, including strains with high thermal resistance.

Most data in the study at hand were from experiments conducted with cultures in the stationary phase. Conditions during culturing of

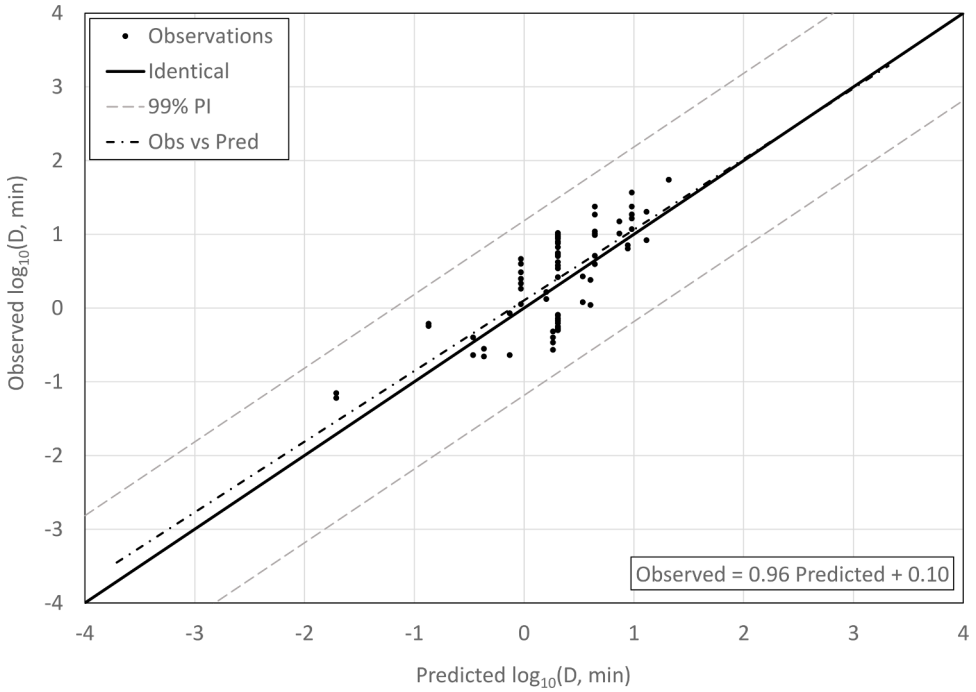

Fig. 10. Observed vs. predicted logD of inactivation of *Cronobacter* spp. (CS, 79 data sets). Predictions were made with the model for inactivation of *S. enterica* using T, a_w, pH and sugar content from the CS data. 99% PI = 99% prediction interval for a single prediction. The formula shows the slope and intercept of the regression line of observed vs. predicted logD (Obs vs Pred).

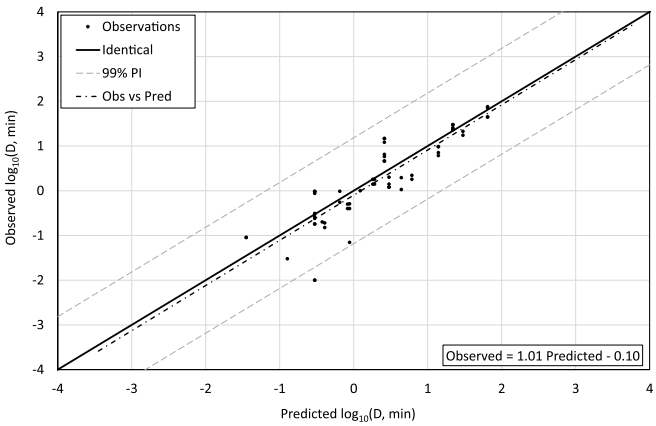

Fig. 11. Observed vs. predicted logD of inactivation of *Yersinia enterocolitica* (YE, 61 data sets). Predictions were made with the model for inactivation of *S. enterica* using T, a_w, pH and sugar content from the YE data. 99% PI = 99% prediction interval for a single prediction. The formula shows the slope and intercept of the regression line of observed vs. predicted logD (Obs vs Pred).

bacteria and the stage of growth (e.g. exponential versus stationary phase) may trigger stress responses including the heat stress response. Therefore, data from experiments with heat shocks were not included in model fitting. In addition to extrinsic factors that can influence heat resistance, different strains of a given species may have intrinsically different heat resistances.

Variability in heat resistance of strains of the same species tested under the same conditions has been demonstrated for various foodborne pathogens, e.g. for *E. coli* (Mercer et al., 2015) and *S. enterica* (Lianou and Koutsoumanis, 2011) and for *L. monocytogenes* (Aryani et al., 2015; Van der Veen et al. 2009). In a detailed study, Aryani et al. (2015) quantified experimental, reproduction, and strain variability in heat resistance using 20 strains of *L. monocytogenes*, which showed that strain variability was much larger than reproduction variability, which was in turn larger than experimental variability. Strain variability can be attributed to differences in the genetic make-up of different strains of the same species. In *L. monocytogenes*, for instance, the presence of a plasmid carrying the gene encoding for the heat shock protein ClpL leads to increased heat resistance of cells (Pöntinen et al., 2017). Another example in *L. monocytogenes* is a mutation in the gene encoding the heat-shock repressor CtsR, leading to increased heat resistance (Karatzas et al., 2003). In the case of *E. coli* and *S. enterica*, a major determinant in the heat resistance of cells is the presence of a locus of heat resistance, encoding several putative heat-shock proteins, proteases, and transport proteins (Mercer et al., 2015, 2017). Clearly, variability in heat resistance can have many different causes, some of which due to biological responses.

Suggested generic predictive model

The proportion of fail-dangerous predictions over the 99%-prediction interval for all species is 2% (expected 0.5%). Adjusting the predictive model (Eq. (2) with coefficients from Table 1) with results from validation for species with $N > 60$ from Table 2 results in Eq. (5).

Where (see Table 2):

- $\beta_{0val} = 0.22$ logD, the validation regression intercept β_0 for species with $N > 60$, suggested as a required safety measure.
- $\beta_{1val} = 0.86$, the validation regression slope β_1 for species with $N > 60$. It is an optional safety measure for negative logD.
- $RSE_{fitted} = 0.4580$, the residual standard error of the predictive model (Table 1).
- T_{PI} = the margin of the prediction interval, expressed as the standardized residual of the predictive model, following a T-distribution with d.f. = 513. $T_{PI95} = 1.965$ and $T_{PI99} = 2.585$. An acceptable margin should be a risk management decision, based on a risk assessment including the probability distributions of the thermal resistance of strains common to the product to be heated and the effect of survival of the heating step.

With the constant increase with $\beta_{0val} = 0.22$ logD alone, the proportions of the observed logDs above the 99%-prediction interval of the model already are close to the expected 0.5% or lower, except for vegetative cells of *C. perfringens* (possibly due to spore formation in experiments) and *S. aureus* (for which higher resistance has been reported), as discussed above.

The model is applicable within the observed ranges of T, a_w, pH and sugar content (see Methods section and Figs. 1 through 4) and should include an assessment and acceptation of a risk of exceeding the upper limit of a chosen prediction interval.

Application of the model with $\beta_{0val} = 0.22$ logD to pasteurization of milk (aw 0.993, pH 6.5, sugar content 0.046 m/m) at 76 °C would result in a D of 1.34 s at the upper limit of the 95% prediction interval. This D would be 3.27 s when applying the optional safety measure of multiplying logD with $\beta_{1val} = 0.86$ before adding 0.22 logD and the margin of the prediction interval.

$$\log D_{(min)} = \beta_{0val} + \beta_{1val}\left(10.23 - 0.1568\,T_{(°C)} - 33.25\,a_w + 32.02\,a_w{}^2 + 0.1776\,pH + 1.484\,Sugar_{(m/m)} + 0.5599\,T_{(°C)}a_w - 0.5754T_{(°C)}a_w{}^2\right) + T_{PI}RSE_{fitted} \quad (5)$$

Conclusions

A predictive model for thermal inactivation of vegetative bacteria was based on data from experiments in a number of different foods and media with just *S. enterica*. The model adjusts the inactivation effect of temperature for the effects of a_w, sugar and pH. The model, validated with data from a large variety of genera, species and strains (including heat sensitive and heat resistant strains), heated in a variety of foods and media reported in literature, predicts the inactivation of vegetative bacteria of other genera and their species surprisingly well. The variability of thermal resistance within species has been shown to be larger than the variability between genera, based on the precision of the validations:

- The similarity of the variability of logD in the validations and in the prediction model.
- The small standard deviation of intercepts (constant differences in means) of logD of species compared to the overall standard deviation of prediction errors.

A further decrease of the RSE and therefore the width of the logD prediction interval most likely is possible by adjusting for other characteristics of the heating media or food products and for conditions of culturing, isolation, storage, inoculation, heating/cooling, and enumeration.

The evaluation of the predictive model for seven of the species combined shows:

- A systematic underestimation of logD with 0.22 (expected 0);
- A prediction bias with a slope of 0.86 (expected: 1), indicating:
  - Underestimation of low logD (short heating, usually at higher temperatures);
  - Overestimation of high logD (long heating, usually at lower temperatures).

Both accuracy errors suggest the possibility for improvement by adjusting for between-study variability and the effects of other differences in composition of heating media or food products, heating conditions and culturing conditions.

After an increase of the predicted logD with 0.22, the proportions of the observed logDs above the 99%-prediction interval of the model are close to the expected 0.5% or lower for most genera. Multiplying negative logD's with 0.86 before adding 0.22 to logD would be an extra safety measure to consider.

The findings suggest that the presented predictive model is applicable for designing – to be followed by validation - the thermal inactivation of a variety of vegetative pathogenic foodborne bacteria common to the food chain.

Author statement

The authors do not wish to describe their exact roles, all have contributed in their own way.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mran.2021.100174.

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