Multiple regression model for thermal inactivation of *Listeria monocytogenes* in liquid food products

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**A B S T R A C T**

A multiple regression model was constructed for thermal inactivation of *Listeria monocytogenes* in liquid food products, based on 802 sets of data with 51 different strains and 6 cocktails of strains published from 1984 to 2010. Significant variables, other than inactivation temperature, were pH, sodium chloride content, sugar content, the temperature of growth or storage before inactivation, in addition to a heat shock before inactivation. The constructed model for thermal inactivation of *L. monocytogenes* has a reduced variability as these variables are known to influence the thermal resistance (and these are known or controllable in practice). Mean simulation results of inactivation of *L. monocytogenes* during pasteurisation (20 s, 76°C) of raw milk (calculated mean level after growth 14 cfu/l) were comparable with results of a single regression model constructed from inactivation data found in experiments in milk only (175 data sets, 18 strains/cocktails). Both models predicted a probability of survival of less than 1 in a billion litres. The study shows that multiple regression modelling can be used to obtain a model from all data available, with a limited and realistic uncertainty level, while retaining the variability of heat resistance due to the 51 strains and 6 cocktails of strains (unknown and not controllable in practice).

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1. Introduction

*Listeria monocytogenes* can cause listeriosis, a severe illness with a high mortality rate, especially in vulnerable parts of populations, e.g. unborn children, elderly and immune-deficient people (*World Health Organization, 2004*, p. 269). *L. monocytogenes* is very capable of growth in some food products, even when stored at refrigeration temperatures. For such products, *Listeria* needs to be inactivated, e.g. by thermal processing, and the probability of survival of a single cell needs to be very low. The variability of the efficacy of thermal inactivation of *L. monocytogenes* (e.g. during pasteurisation) can be estimated using a single linear regression model that is based on publicly available literature data, not taking into account effects of variables other than inactivation temperature and holding time (*Van Asselt & Zwietering, 2006*).

When calculating inactivation of *L. monocytogenes* for a specific food and specific processing conditions in Monte Carlo simulations, this model is likely to overestimate the variability of the thermal inactivation efficacy, as the model only takes into account the effect of temperature and holding time. Factors that contribute to increased variability include differences in food composition (pH, NaCl etc.), processing conditions (e.g. those before heating), bacterial strain characteristics (intra-species variability) and other variables that have been shown to influence thermostolerance of *L. monocytogenes* (*Doyle, Mazotta, Wang, Wiseman, & Scott, 2001*). *L. monocytogenes* has been shown to be more heat-sensitive in, for example, apple juice (pH 4, *Mazotta, 2001*) than in raw milk (pH 6.6, *Doyle et al., 2001*) and less sensitive in liquid egg products with NaCl or sugar than in liquid egg products without these additives (*Palumbo, Beers, Bhaduri, & Palumbo, 1995*). The variance of the single, unadjusted predictive regression model based on measurements in all sorts of media and food products is too high to realistically predict variability of inactivation in a specific product under specific processing conditions.

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However, restriction to a certain food group or product and fixed processing conditions may lead to underestimation of the variability of thermostolerance as inactivation data for single food groups or products are available only for a limited number of L. monocytogenes strains (Den Besten & Zwietering, 2012). The objective of this research was to generate a multiple regression model that can predict (variability of) thermal inactivation for a maximum variety of strains from literature data while accounting for effects of food composition and processing conditions. Thus, the variance in the predictive inactivation model is limited to the variance due to uncertainty about the strain present in the raw product. As specific data on food composition are lacking in most reported studies on heat inactivation in solids (fish, seafood, meat, vegetables), the model was limited to fluid foods and fluid media. A preliminary version of the model was presented at the Seventh International Conference on Predictive Modelling in Foods in Dublin (Van Lieverloo, De Roode, Fox, Zwietering, & Wells-Bennik, 2011).

2. Materials and methods

Heat inactivation data according to the Bigelow model (Bigelow, 1921) and some condition variables were present in a database constructed from literature as described in a meta-analysis by Van Asselt and Zwietering (2006). Data on more variables were collected from papers cited in the latter paper (Casadei, Esteves de Matos, Harrison, & Gaze, 1998; Chhabra, Carter, Linton, & Cousin, 1999; Doyle et al., 2001; Holsinger, Smith, Smith, & Palumbo, 1992; ICMFS, 1996; Mazzotta, 2001) and 37 original papers from the reviews of ICMFS (1996) and Doyle et al. (2001).

The database was further supplemented with other and more recent literature on thermal inactivation in fluid foods (Edelson-Mammel, Whiting, Joseph, & Buchanan, 2005; Hassan, Álvarez, Raso, Condón, & Pagán, 2005; Hassan, Manas, Pagán, & Condón, 2007; Hassan, Manas, Raso, Condón, & Pagán, 2005; Huang, 2004; Ignatova, Leguerinel, Guilbot, Prévost, & Guillot, 2007; Juneja & Eben, 1999; Maisnier-Patin, Tatini, & Richard, 1995; Van der Veen, Wagendorp, Abeel, & Wells-Bennik, 2009).

Five groups of liquid heating menstrua were distinguished: Dairy (n = 260: milk, cream, butter and ice-cream), liquid eggs (n = 107: whole, white and yolk), gravy (n = 87: beef and chicken), fruit and vegetable juices (n = 32: apple, orange, white grape and cabbage) and liquid media (n = 321: deionised water, physiological saline, phosphate buffer, brain heart infusion, tryptose phosphate broth and trypticase soy broth (with or without yeast extract)). In total, 807 data sets were collected with the following 13 variables:

- Inactivation temperature (50–80 °C) and 10log of holding time (9.59·10^{-4} – 356 min) needed for 10-fold inactivation (D). The maximum coefficient of log-linear heat inactivation was calculated from raw data in the case that only coefficients for non-log–linear inactivation were provided;
- Heating method. Method 1 = lab scale pasteuriser with flow (n = 93), method 2 = low culture volume in large volume pre-heated menstruum (n = 211), method 3 = low volume in submerged glass capillary tube or coil (n = 366), method 4 = large volume in glass vial in water bath (n = 137);
- pH (n = 807, 5.0–9.3), sodium chloride (NaCl, n = 760, 0.033–20%), fat (n = 518, 0.1–83%), sugars (n = 456, 0.2–58%) and lactate (n = 33, 0.07–0.8%);
- Temperature (n = 807, 0–43 °C) and duration (n = 807, 0.25–336 h) of last temperature phase (excluding heat shock);
- Heat shock (yes/no) and the temperature difference with last phase before shock (n = 54, 7–41 °C);
- Adaptation to sodium chloride (n = 6) or acid (n = 56).

Missing data on pH and concentrations of fat, salt and sugars in growth media, dairy, juices and egg (parts) were estimated from other literature (pH of gravy: Huang, Yousef, Matthews, & Marth, 1993) or the internet (see list of websites consulted, following the list of literature references). Data sets (26) with antimicrobials were not included: peroxide (Kamau, Doores, & Pruitt, 1990; Lou & Yousef, 1996; Palumbo, Beers, Bhaduri, & Palumbo, 1996), lactoperoxidase (Kamau et al., 1990), nisin (Knight, Bartlett, McKellar, & Harris, 1999; Maisnier-Patin et al., 1995) and ethanol (Lou & Yousef, 1996). In total, the 807 data sets from 53 papers included 51 L. monocytogenes strains and 6 cocktails of strains. 279 data sets involved experiments with strain Scott A, 118 data sets involved experiments with cocktails of strains.

3. Theory and calculation

3.1. Model construction

Weight to volume fractions (wt/vol) of fat, sodium chloride, sugars and lactate were 10log transformed (10log(p/(1 – p))) to warrant linearity and a constant variance with a close to normal distribution of the residuals of these regression submodels. Zero values were transformed to the decimal below the lowest value, i.e. –5 for 10log(wt/vol sodium chloride) and –4 for 10log(wt/vol fat, sugar and lactate). The duration of the last temperature phase (0–336 h, culturing or storage, excluding heat shock) was 10log transformed. Statistical analysis was performed using GenStat 14.1 (VSN International Ltd.). The highest colinearities that were found were: 1) between 10log(sodium chloride) and 10log(fat), with a correlation coefficient (CC) of –0.38 and 2) between the duration and temperature of the last temperature phase excluding heat shock (CC = –0.39). The ‘all-subsets regression’ procedure (selecting the best combination of significant model variables) of GenStat was used to obtain the primary model (without interaction terms) with significant variables (p < 0.05). Non-linear submodels (quadratic and cubic) were tested for all continuous variables. As variables may interact (the regression coefficient of one variable being dependent on the value of another variable) the following interaction terms were tested separately:

- Inactivation temperature (with all other variables, see Section 2);
- All possible pairs of pH, 10log(sugar), 10log(sodium chloride = NaCl) and 10log(fat);
- pH and acid adaptation;
- 10log(NaCl) and NaCl adaptation;
- Heat shock (yes/no), heat shock temperature difference and duration and temperature of the last temperature phase.

3.2. Graphical presentation of the models

In the graphs with multiple regression models, the residual variance of the adjusted 10logD is shown: Figs. 2, 3 and 5 for instance demonstrate the effect of multiple adjustments on the variance of 10logD versus inactivation temperature. The 10logD values in graphs 2–5 were adjusted only with the sum of the products of the regression coefficients and the mean value of continuous variables, e.g. pH or 10log(NaCl). No adjustments to
the $10\log$-values were made in these graphs for binomial or multinomial variables (e.g. heat shock or heating method). In the predictions of heat inactivation in milk, adjustments for all variables were applied.

### 3.3. Simulation of growth and heat inactivation in milk

The variability of heat inactivation of *L. monocytogenes* during pasteurisation of milk was simulated by Monte Carlo analysis (Vose, 2008) using Microsoft Excel and the Palisade @Risk add-in (Palisade Inc.). Monte Carlo analysis was applied to calculate heat inactivation kinetics with probability distribution functions (pdfs) of data per variable instead of calculating with single data values. The inactivation was calculated by multiplying the regression coefficients per variable of the selected model with the uniform pdfs of the corresponding variables with a given range (e.g. pH 6.5–6.7). For prediction of the $10\log D$ for specific food products and processing conditions, the standard error of the multiple regression model of $10\log D$ was used as the standard deviation of the normal distribution of the model. To simulate growth during storage of raw milk before pasteurisation, the Gamma model (Zwietering, Wit, & Notermans, 1996) was used, with a $\mu_{max}$ of 2 h$^{-1}$, $T_{min}$ of −1.5 °C, $T_{opt}$ of 37 °C, pH$_{min}$ of 4.4, pH$_{opt}$ of 7, water activity (a$_w$) of 0.92, assuming an a$_w$ for milk of 0.993 (Te Giffel & Zwietering, 1999).

### 4. Results and discussion

#### 4.1. Single regression model

Fig. 1 shows the variability of the $10\log D$ of the 807 data sets plotted against inactivation temperature ($T$), showing the variability for 5 food groups. The explained variance ($R^2$) of this single regression model ($log D = 9.07 − T/6.78$, so $log D_{76 °C} = −2.14$ and z = 6.78) is 71.8% and the standard error (s.e.) = 0.471. Based on Fig. 1 and warnings from the statistical software it was concluded that the variance is not equal throughout the temperature range (heteroscedasticity), indicating room for improvement.

#### 4.2. Basic multiple regression model

The variance of thermotolerance of *L. monocytogenes* can be limited by including the effect of variables other than temperature in the inactivation model; this also contributes to a more constant...
variance throughout the temperature range. To select variables for the basic multiple regression model including processing conditions and menstruum composition, all possible combinations of the 14 available explanatory variables (including menstruum group) were tested. This included leaving out one or more variables (without non-linear submodels and interaction terms). The variables salt adaptation ($p = 0.46$), acid adaptation ($p = 0.68$), and $10\logit(lactate)$ ($p = 0.10$) had no significant effect below the $p = 0.05$ level, and therefore these variables were omitted. To limit the complexity of the initial model, five menstruum groups were distinguished (dairy, liquid egg, meat gravy, fruit and cabbage juice, and laboratory media), rather than 20 individual menstrua. Variability of $10\log D$ was limited by accounting for effects of variables other than temperature on inactivation. The variance at 60–70 °C remained too high compared with the variance at lower and higher temperature, resulting in a model still showing heteroscedasticity, having 16 coefficients, with an $R^2$ of 83.2% and a standard error of 0.364 (Fig. 2, model A in Table 1).

### 4.3. Multiple regression model with constant variance of $\log D$ vs. temperature

High $10\log D$ values at the mid (60–70 °C) temperature range were linked to 5–20% sugar and/or 6–10% sodium chloride added to liquid egg products. Low $10\log D$ values in this temperature range were linked to long cold storage in chicken gravy. The presence of the linear terms of $10\logit(NaCl)$, $10\logit(sugars)$ and $10\log(duration$ of last temperature phase) in model A could not reduce this high variance at mid temperatures. The effects of variables may however not be linear but quadratic or cubic. Including quadratic and cubic non-linear submodels for all three variables in model A led to a considerable improvement, also rendering the effect of the temperature of the last temperature phase to be highly significant again ($p < 0.001$). Variability of $10\log D$ was limited by taking into account the effects of variables other than temperature on inactivation. This model B had

### Table 1

Coefficients (and standard error) of models of the effect of inactivation temperature and other variables on $10\log D$ (D – time in minutes for 10-fold inactivation). Significance levels are $p < 0.001$ unless indicated otherwise: **$p < 0.01$, *$p < 0.05$, & $p > 0.1$.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Single regression models</th>
<th>Multiple regression models</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All fluids</td>
<td>Milk alone</td>
</tr>
<tr>
<td>Intercept</td>
<td>9.07 (0.198)</td>
<td>10.0 (0.225)</td>
</tr>
<tr>
<td>Inactivation temperature (°C)</td>
<td>$-0.148$ (0.0033)</td>
<td>$-0.162$ (0.0036)</td>
</tr>
<tr>
<td>$\logit$ (sugars wt/vol)$^a$</td>
<td>0.125 (0.018)</td>
<td>0.381 (0.109)</td>
</tr>
<tr>
<td>$\logit$ (sugars wt/vol)$^b$</td>
<td>0.077 (0.016)</td>
<td>0.038 (0.017)*</td>
</tr>
<tr>
<td>$\logit$ (fat wt/vol)$^c$</td>
<td>0.104 (0.021)</td>
<td>5.67 (0.429)</td>
</tr>
<tr>
<td>$\logit$ (NaCl wt/vol)$^d$</td>
<td>0.217 (0.023)</td>
<td>0.237 (0.023)</td>
</tr>
<tr>
<td>Last temperature phase (°C)$^b$</td>
<td>7.45 $10^{-3}$ (1.17 $10^{-3}$)</td>
<td>6.18 $10^{-3}$ (1.29 $10^{-3}$)</td>
</tr>
<tr>
<td>$\log$ (last temp. phase (h))$^b$</td>
<td>$-0.252$ (0.037)</td>
<td>0.293 (0.079)</td>
</tr>
<tr>
<td>Heat shock difference (°C)$^b$</td>
<td>0.0146 (0.0023)</td>
<td>$-0.130$ (0.030)</td>
</tr>
<tr>
<td>Heat shock (yes/no)$^b$</td>
<td>0.049 (0.049)</td>
<td>0.483 (0.048)</td>
</tr>
<tr>
<td>Heating method 2$^d$</td>
<td>$-0.130$ (0.054)*</td>
<td>$-0.193$ (0.045)</td>
</tr>
<tr>
<td>Heating method 3$^d$</td>
<td>$-0.103$ (0.046)*</td>
<td>$-0.187$ (0.035)</td>
</tr>
<tr>
<td>Heating method 4$^d$</td>
<td>0.072 (0.057)$^b$</td>
<td>$-0.130$ (0.048)$^b$</td>
</tr>
<tr>
<td>Liquid egg$^e$</td>
<td>0.201 (0.064)$^f$</td>
<td>0.348 (0.092)</td>
</tr>
<tr>
<td>Meat gravy$^e$</td>
<td>0.383 (0.074)</td>
<td>0.466 (0.116)</td>
</tr>
<tr>
<td>Cabbage / fruit juice$^e$</td>
<td>0.337 (0.094)</td>
<td>$-0.501$ (0.077)</td>
</tr>
<tr>
<td>Media$^e$</td>
<td>0.176 (0.058)$^f$</td>
<td>0.138 (0.091)$^f$</td>
</tr>
<tr>
<td>Estimated standard error</td>
<td>0.471</td>
<td>0.277</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.718 %</td>
<td>92.3 %</td>
</tr>
<tr>
<td>Number of data sets</td>
<td>807</td>
<td>175</td>
</tr>
<tr>
<td># of coefficients</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

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$^a$ As fractions.

$^b$ Duration and temperature of last temperature phase, either during culturing or storage, not heat shock.

$^c$ Heat shock (54 data sets), the temperature difference is with the last temperature phase (culturing or storage).

$^d$ Heating method 1 – lab scale pasteuriser with flow (n = 93), 2 – low culture volume in large volume pre-heated menstruum (n = 211), 3 – low volume in submerged glass capillary tube or coil (n = 366), 4 – large volume in glass vial in water bath (n = 137). Reference method is heating method 1.

$^e$ Reference menstruum is dairy.
an $R^2$ of 89.1% and a standard error of 0.294 (having 20 coefficients) and is presented in Table 1 and Fig. 3. Differences between heating methods and between menstruum groups became more significant when changing from model A to model B.

Inclusion of quadratic and cubic terms for other variables and interaction terms were investigated. In many cases submodels were found that had significant effects, however the increase of the $R^2$ and the decrease of the s.e. were marginal in those cases.

4.4. Optimum model

An optimal model was considered to have less variables than model B while retaining a high descriptive power, expressed as the $R^2$. The cubic submodel of $\text{logit(sugar)}$ could be omitted without changing the $R^2$ and s.e. too much, leaving a model with all variables and submodels highly significant ($p < 0.001$, except the difference between heating methods 1 and 4, $p = 0.008$). The inclusion of $\text{logit(NaCl)}$ was considered necessary and a protective effect of sodium is consistent with various individual reports (Edelson-Mammel et al., 2005; Jorgensen, Stephens, & Knochel, 1995 and Juneja & Eblen, 1999). The linear or linear – quadratic submodels alone were incapable of reducing variance. As the 4th power polynomial model of the adjusted $\text{logD}$ versus $\text{logit(NaCl)}$ followed the experimental data at low and high NaCl concentrations much better than the 3rd polynomial model, the 4th order submodel of $\text{logit(NaCl)}$ was included (see Fig. 4, the slight undulation of the line without a common experimental design that was focussed on multiple regression analysis, these modelling uncertainties could be expected. For the applicability of the model, this means that the effect of some secondary variables may not be estimated correctly for all possible fluids. Therefore, a comparison of the results of the multiple regression model with results from a single regression model of specific fluids and processing conditions remains necessary. Fig. 4 also shows that the level of variance of the effect of $\text{logit(NaCl)}$ on the adjusted $\text{logD}$ is higher than the level of variance of the effect of temperature (Fig. 5). This is the case for other adjusting variables as well.

4.5. Comparison with single regression models

Model C was used to predict inactivation during pasteurisation of milk (76 °C, 20 s, no previous heat shock) and the results were compared with predictions based on the single, unadjusted regression model presented in section 4.1 and those based on a single regression model of milk data only (175 data sets 0–4.5% fat, with pH at 6.5 or 6.6, no previous heat shock, 18 strains or cocktails of strains, 4 high standardised residuals, 1 warning of high leverage, see Table 1). The assumed milk and processing characteristics are presented in Table 2. The mean $D$ during pasteurisation at 76 °C was 0.222 s, resulting in a mean $\text{logD}$ inactivation of 90.2 at 20 s holding time, compared with a mean of 46.0 that was predicted using the single regression model of all fluids, and a mean of 74.4 that was predicted using the single regression model of milk data alone (Table 2). The multiple regression model C at 76 °C nevertheless is more conservative in its prediction of inactivation than the single regression model with milk data alone, as model C has a larger standard error (0.302 versus 0.277). The higher level of uncertainty in model C results in a lower $\text{logD}$ inactivation during 20 s at the lower $p = 1 \times 10^{-6}$ percentile of the pdf (a probability of 1 in a million that $\text{logD} = 3.3$ for model C versus 3.8 for the single regression model, Table 2). The single, unadjusted regression model of all fluids has an even more conservative mean and lower $p = 1 \times 10^{-6}$ percentile (46.0 and 0.3 respectively), but is likely to underestimate the actual inactivation due to the presence of unadjusted effects of secondary variables.

4.6. Simulation of heat inactivation in raw milk

Sanaa, Corroler, and Cerf (2004) estimated mean concentrations of $L.\$\text{monocytogenes}$ in raw milk from two areas in France at 0.3 and 0.8 cells/ml, with their mean being 0.55 cells/ml. Assuming a distribution of the concentration of Gamma (1; 0.55) cells/ml in raw milk, the mean concentration after growth during cold storage was estimated to be 14 cells/ml. The P99.9999 in raw milk was approximately 13 cells/ml, and after growth during cold storage approximately 3500 cells/ml (16–80 h, 1 million iterations). The $\text{logD}$ for inactivation of $L.\$\text{monocytogenes}$ at pasteurisation conditions was simulated in a Monte Carlo analysis with the three models compared in Section 4.5. To include variability in milk composition, the following ranges were assumed for the uniform distributions of the model variables: pH 6.5–6.7; sugar 4.5–4.7%; NaCl 0.2–0.3%; temperature during cold storage 5–7 °C.
The Monte Carlo simulation with the single, unadjusted regression model of all fluids resulted in a probability of 4.92 × 10⁻⁴ (SD 2.04 × 10⁻⁵ of 10 simulations of 1 million iterations each) of the presence of a surviving L. monocytogenes cell in a litre of milk (which is 1 in approximately every 2000 L). The results of this worst case simulation are unrealistic. With the single regression model and the presence of a surviving L. monocytogenes in 10 simulations of 1 million iterations each. The means of the estimated lower p = 1 × 10⁻⁶ percentile of the 10log inactivation during these 10 simulations were 0.21 (SD 0.03) for the single regression model of all fluids, 3.49 (0.22) for the single regression model of milk data alone and 3.00 (SD 0.20) for the multiple regression model C of all fluids. Therefore, the probability of survival of L. monocytogenes during pasteurisation of raw milk at 76 °C during 20 s can be estimated with model C at less than 1 in a billion (10⁹) litres.

5. Conclusions

A multiple regression model was created from literature data to predict heat inactivation of L. monocytogenes in fluids such as dairy (milk, cream, butter), meat gravy, fruit and vegetable juices and liquid eggs with or without added sugar and sodium chloride. The model includes the full variability of strain tolerance to heat and limits the variability due to differences in food composition (pH, sodium chloride, sugar) and processing conditions (storage temperature, heat shock). The model shows the power of meta-analyses, the relative effects of variables other than temperature and the uncertainties of predicting heat inactivation with data from a limited number of strains.

As the model is constructed from data that have been published in a period of more than 25 years without a common experimental design, the influence of some variables is not known for all conditions or tested with similar intensity. Therefore, the results of the model are not certain enough to design heat inactivation processes without comparing the mean results of the multiple regression model with the mean results of the single regression models for the specific liquid. We recommend to use the variability of the multiple regression model (0.302 /C0) to calculate the variability due to uncertainty about the L. monocytogenes strains present in the food.

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### Appendix A. Supplementary material

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.foodcont.2012.05.078.

### References


