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

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Abstract

A model was constructed from literature data for thermal inactivation of *Listeria monocytogenes* in liquid food products based on 735 sets of literature data. Significant variables were pH, sugar and fat content and the time and temperature of growth or storage before inactivation, as well as a heat shock. The model reduces the variability in the dataset due to these variables (known or controllable in practice), while keeping the variability of heat resistance of the 58 strains (unknown and not controllable in practice).

Keywords: pasteurisation, food matrix, processing, Monte Carlo analysis

Introduction

The variability of the efficacy of thermal inactivation of *L. monocytogenes* (e.g. during pasteurisation) can be estimated by a model that is based on literature data. Differences in food composition, process conditions and other variables can influence thermal inactivation. When calculating inactivation of *L. monocytogenes* for a specific food and process using Monte Carlo simulations, there is likely an overestimation of the variability of the thermal inactivation efficacy. On the other hand, using inactivation data in a certain food based on a limited number of *L. monocytogenes* strains may lead to underestimation of the variability of strain resistance to heat. The objective of this research was to generate a multivariate regression model to predict (variability of) thermal inactivation from literature data while accounting for effects of food composition and processing conditions. As specific data on food composition is lacking in most literature on heat inactivation in solids (fish, sea food, meat, vegetables), the model was limited to fluids.

Materials and Methods

Inactivation data and some condition variables were present in a database constructed from literature as described by Van Asselt & Zwietering (2006). Data on more variables were collected from the original papers they cited and from the cited reviews of ICMSF (1996) and Doyle *et al*. (2001). The database was further supplemented with other, mostly more recent literature (Edelson-Mammel *et al*. (2005), Hassani *et al*. (2005a, 2005b, 2007), Huang (2004), Ignatova *et al*. (2007), Juneja & Eblen (1999), Maisnier-Patin *et al*. (1995), Van der Veen *et al*. (2007)). Missing data on pH and concentrations of fat, salt and sugars in growth media, dairy, juices and egg (parts) were estimated from other literature or the internet. Data sets (26) with antimicrobials (peroxide, lactoperoxidase, nisin and ethanol) were not included. In total, the 801 data sets from 53 papers included 58 *L. monocytogenes* strains or cocktails (7).
Statistical analysis was performed using GenStat 13.2 (VSN International Ltd.). Concentrations of fat (0 – 83%), sodium chloride (0 – 20%) and sugars (0 – 58%) were 10-logarithmically transformed to approach a normal distribution, as was the duration of the last temperature phase (0 – 336 h, culturing or storage, excluding heat shock). Zero values were transformed to -5 (% sodium chloride), and -4 (% fat and sugar). The highest, acceptable, colinearity found was between ^{10}log(sodium chloride) and ^{10}log (fat), with a correlation coefficient of 0.24 (-0.06 when zero values were excluded). The 'all-subsets regression' procedure was used to attain the basic linear model without interaction terms.

Results and Discussion

Preliminary multivariate modelling could not reduce unequal variance over the temperature range, the variance at 60 – 70 °C remained too high. High ^{10}logD (D = time to 10-fold reduction) was linked to 5-20% sugar and/or sodium chloride added to liquid egg products. Low ^{10}logD was linked to long cold storage in chicken gravy. As inclusion of ^{10}log(sodium chloride), ^{10}log(sugars) and ^{10}log(duration of last temperature phase) in the model could not reduce this high variance at mid temperatures, chicken gravy data (40 data sets) were removed from the data set, as were liquid egg products with added sugar or sodium chloride (26 sets). This limited the concentration range of sodium chloride to a maximum of 8.8% (initially 20%), but stabilised the variance. Figure 1 shows the variability of all ^{10}logD values, not corrected for food or process variables. The univariate model of 735 datasets (logD = 9.07 – T / 6.74) had an R^2 of 0.77 and a standard error of 0.409.

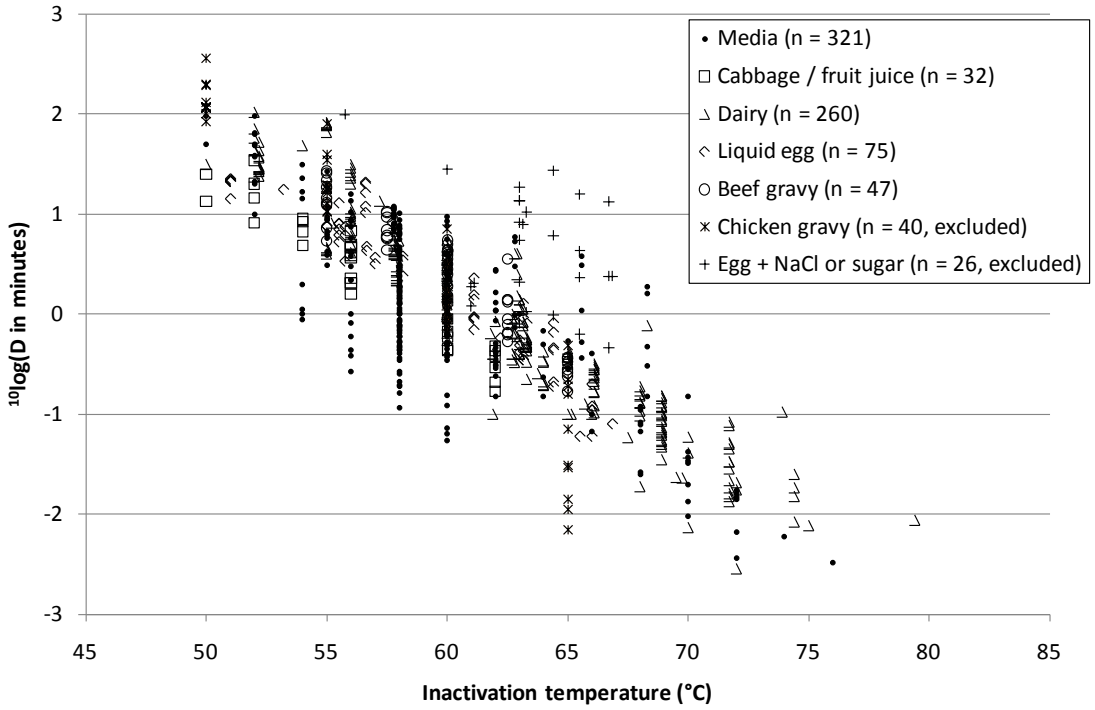

Figure 1: Variability of inactivation times (D = time to 10-fold reduction) of Listeria monocytogenes per heating menstruum as a function of the heating temperature. Inactivation times are not corrected for effects of other variables. Total number of data sets is 801 and 66 of these were excluded for further modelling (chicken gravy and liquid eggs with added sugar or sodium chloride). Fruit juices are apple, orange and white grape. Dairy includes milk, cream, butter and ice-cream. Liquid eggs are separated in whole, white and yolk. Media include deionised water, physiological saline, phosphate buffer, brain heart infusion, tryptose phosphate broth and trypticase soy broth (with or without yeast extract).

To select variables for the basic multivariate model including processing conditions and menstruum composition, all possible combinations of variables were tested, including leaving out one or more variables. To limit the complexity of the initial model, individual menstrua (17 groups) were not included at first. The selected best model had an R^2 of 88.3% and a standard error or 0.292 and is presented as model A in Table 1. Allowing for interaction between heating temperature and menstruum groups, i.e. allowing different slopes per menstruum group, did not change R^2 or standard error, and consequently interaction terms were not included. ^{10}log(sodiumchloride) was not significant (p = 0.055), had little effect on R^2 (88.4%) and the standard error (0.292) and was not included in model A.

Table 1: Coefficients (and standard error) of models of the effect of heat and other variables on 10log D (D = time in minutes for 10-fold inactivation). Significance levels are $p < 0.001$ unless indicated otherwise: ** $p < 0.01$, * $p < 0.05$, # $p < 0.1$, & $p > 0.1$.

Variable	Model A	Model B
Intercept	**9.33** (0.189)	**9.01** (0.170)
Heating temperature (°C)	**-0.161** (0.0025), z = 6.21	**-0.157** (0.0023), z = 6,37
pH	**0.165** (0.011)	**0.167** (0.011)
^{10}log(sugars % wt/vol)	**0.081** (0.018)	**0.090** (0.017)
^{10}log(fat % wt/vol)	**0.063** (0.014)	**0.060** (0.014)
Last temperature phase (°C) [a]	**0.0053** (0.009)	**0.0060** (0.009)
^{10}log (last temp. phase (h)) [a]	**-0.220** (0.028)	**-0.249** (0.027)
Heat shock difference (°C) [b]	**0.0153** (0.018)	**0.0138** (0.018)
Heating method 2 [c]	**-0.189** (0.047)	
Heating method 3 [c]	**-0.078** (0.037)*	
Heating method 4 [c]	0.078 (0.049)&	
Liquid egg [d]	**-0.142** (0.064)*	-0.074 (0.060)&
Beef gravy [d]	**0.516** (0.073)	**0.414** (0.073)
Cabbage / fruit juice [d]	**0.216** (0.077)**	0.116 (0.071)&
Media [d]	0.071 (0.051)&	0.069 (0.050)&
Estimated standard error	**0.292**	**0.298**
R^2	**88.3%**	**87.8%**
Number of data sets	735	735

[a] Duration and temperature of last temperature phase, either during culturing or storage, not heat shock.
[b] Heat shock (54 sets) temperature difference with the last temperature phase (culturing or storage).
[c] 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 = 350), 4 = large volume in glass vial in water bath (n=81). Reference method is heating method 1.
[d] Reference menstruum is dairy

When all 17 individual menstrua were included in model A (instead of menstruum groups), milk, cream and some media were significantly different from other menstrua, R^2 was 89.9% and standard error 0.271. Whereas there could be merits in considering all menstrua separately, doing so would result in considerable increase of model complexity and general applicability, which is undesirable. Allowing polynomial effects of variables and interaction between variables in model A, the model would improve slightly (R^2 = 89.4, s.e. = 0.278), the polynome of ^{10}log(sodium chloride) would be included, as would the product of ^{10}log(sodium chloride) and ^{10}log(sugars). In this model, however, an increase of the ^{10}log(sodium chloride) terms would have a lowering effect on logD and this is contradictory to results in individual papers (Jorgensen *et al.* (1995), Juneja & Eblen (1999) and Edelson-Mammel *et al.* 2005). Furthermore, these changes would result in a lower and more uncertain intercept, only a low increase of R^2 and low decrease of the estimated standard error, as well as in increased complexity. Therefore, this change is suboptimal and model A is preferred. An even simpler model with a relatively high R^2 and low standard error also excludes the effect of the heating method from model A, resulting in model B (Table 1). This model is overall preferred, as the effect of heating method does not seem to follow logic; the best heating and cooling method (1: lab scale pasteuriser with flow) gives results that are not significantly different from the worst heating and cooling method (4: large volume in water bath). Model B is applied for inactivation of *L. monocytogenes* in raw milk (without pre-heating, i.e. no heat shock), described in Formula 1 (standard errors are given in Table 1).

$$^{10}\log D_{\text{raw milk}} = 9.01 - 0.157 \text{ heating temperature (76 °C)} + 0.167 \text{ pH (6.5 - 6.7)} + 0.090\ ^{10}\log(\text{sugar (4.5 - 4.7\%)}) + 0.060\ ^{10}\log(\text{fat (3.8 - 4.2\%)}) + 0.0060 \text{ temperature last storage phase (5-7 °C)} - 0.249\ ^{10}\log(\text{time last storage phase (16 – 80 h)}) \pm 0.298 \quad (1)$$

Sanaa *et al.* (2004) estimated mean concentrations of *L. monocytogenes* in raw milk from two areas in France at 0.3 and 0.8 cells/l, with their mean being 0.55 cells/l. Assuming a distribution of the concentration of Poission(Gamma (1;0.55)) cells/l, the P99.9999 in raw milk is 13 cells/l (10 million iterations). With the univariate model of 735 data sets, uncorrected for the effect of food composition and processing conditions, there is a calculated probability of 5.10^{-5} of the presence of a surviving *L. monocytogenes* cell in a litre of milk pasteurised at 76 °C for 20 s (assuming equal variance at all temperatures). Using the preferred model B, and assuming uniform distributions of variables with ranges described in Formula 1, the calculated probability is reduced to less than 1.10^{-7}, due to the lower variability resulting from the inclusion of the effect of product and process variables.

Conclusion

A practical multivariate regression model from literature data can be used to predict heat inactivation of *L. monocytogenes* in fluids like dairy (milk, cream, butter), fruit and vegetable juices and liquid eggs without additives. The model includes variability of strain tolerance to heat and limits the variability for specific processing conditions and food composition.

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