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Development of a hierarchical Bayesian model to estimate the growth parameters of *Listeria monocytogenes* in minimally processed fresh leafy salads

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ABSTRACT

The optimal growth rate μ_{opt} of *Listeria monocytogenes* in minimally processed (MP) fresh leafy salads was estimated with a hierarchical Bayesian model at (mean ± standard deviation) $0.33 \pm 0.16\ h^{-1}$. This μ_{opt} value was much lower on average than that in nutrient broth, liquid dairy, meat and seafood products ($0.7–1.3\ h^{-1}$), and of the same order of magnitude as in cheese. Cardinal temperatures T_{min}, T_{opt} and T_{max} were determined at -4.5 ± 1.3 °C, 37.1 ± 1.3 °C and 45.4 ± 1.2 °C respectively. These parameters were determined from 206 growth curves of *L. monocytogenes* in MP fresh leafy salads (lettuce including iceberg lettuce, broad leaf endive, curly leaf endive, lamb's lettuce, and mixtures of them) selected in the scientific literature and in technical reports. The adequacy of the model was evaluated by comparing observed data (bacterial concentrations at each experimental time for the completion of the 206 growth curves, mean $\log_{10}$ increase at selected times and temperatures, *L. monocytogenes* concentrations in naturally contaminated MP iceberg lettuce) with the distribution of the predicted data generated by the model. The sensitivity of the model to assumptions about the prior values also was tested. The observed values mostly fell into the 95% credible interval of the distribution of predicted values. The μ_{opt} and its uncertainty determined in this work could be used in quantitative microbial risk assessment for *L. monocytogenes* in minimally processed fresh leafy salads.

1. Introduction

Quantitative microbial risk assessment integrates the prediction of the fate of pathogenic microorganisms in foods. Primary models in predictive microbiology describe, with several parameters, the kinetics of microbial survival or growth in the food. Secondary models describe the effects of environmental factors (temperature, pH, a_w, etc.) on the parameters of microbial kinetics (Ross and Dalgaard, 2004). Among these, cardinal parameter models (CPMs) involve predicting the growth rate in a given condition in terms of (i) a function described by a growth factor "gamma" (γ) that has a value between 0 and 1 and expresses the relative effect of the environmental factors (Zwietering et al., 1992b), and (ii) the value of the growth rate in optimal growth conditions μ_{opt}. Augustin and Carlier (2000) and Augustin et al. (2005) determined cardinal parameters and μ_{opt} values of *Listeria monocytogenes* in dairy products, meat, liquid egg, and seafoods from an extensive literature review.

A method using Bayesian inference was applied by Pouillot et al. (2003) to determine cardinal parameters and μ_{opt} of *L. monocytogenes* in milk. In a Bayesian framework, model parameters are random variables, firstly described by a prior distribution (Gelman et al., 2004; Robert, 2001). These prior distributions are uninformative or vague when information about parameters is absent or poor. Conversely, informative prior distributions may be obtained with expert knowledge or data from previous studies. From a combination of prior distributions and data, posterior distributions over all parameters are computed using Bayes' theorem. In hierarchical Bayesian models, these parameters are themselves defined by distributions that have proper "hyperparameters". In these models hyperprior distributions are assigned to hyperparameters. Through the prior and hyperprior distributions, hierarchical Bayesian models account for both the variability and the uncertainty of the parameters of interest, such as the optimal growth rate μ_{opt} in the present work. The distributions of predicted quantities of interest, such as *L. monocytogenes* concentrations at different temperatures and times, can be computed from the posterior distributions of the parameters.

L. monocytogenes can multiply on a range of vegetables and there are reports of several listeriosis outbreaks following vegetable consumption (Nguyen-the and Carlin, 2000). The aim of this work was to determine using a hierarchical Bayesian model a μ_{opt} value specific to minimally processed (MP) fresh leafy salads, its variability

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Table 1
Source of growth curves showing the fate of *Listeria monocytogenes* in minimally processed fresh leafy salads used in this work.

Source	Type of salad	Range of temperature tested	Number of growth curves
Beuchat and Brackett (1990)	Iceberg lettuce	5 °C and 10 °C	31
Carlin and Nguyen-the (1994)	Lettuce, curly leaf endive, lamb's lettuce, broad leaf endive	10 °C	4
Carlin et al. (1995)	Broad leaf endive	3 °C, 6 °C, 10°C and 20 °C	25
Carlin et al. (1996a)	Broad leaf endive	3 °C and 10 °C	39
Carlin et al. (1996b)	Broad leaf endive	10 °C	3
Delaquis et al. (2002)	Iceberg lettuce	1 °C and 10 °C	4
Farber et al. (1998)	Lettuce	4 °C, 10 °C and 25 °C	3
Francis and O'Beirne (1997)	Lettuce	3 °C and 8 °C	12
Francis and O'Beirne (2001a)	Iceberg lettuce	8 °C	2
Francis and O'Beirne (2001b)	Iceberg lettuce	4 °C and 8 °C	4
Francis and O'Beirne (2005)	Iceberg lettuce	8 °C	15
Garcia-Gimeno et al. (1996)	Broad leaf endive	4 °C	1
Jacxsens et al. (1999)	Lettuce	7 °C	2
Jacxsens et al. (2002)	Broad leaf endive	2 °C, 4 °C, 7 °C and 10 °C	4
Kakiomenou et al. (1998)	Lettuce	4 °C	2
Koseki and Isobe (2005)	Iceberg lettuce	5 °C, 10 °C, 15 °C, 20 °C, and 25 °C	5
Li et al. (2002)	Iceberg lettuce	5 °C and 15 °C	10
Nguyen-the et al. (1996)	Broad leaf endive	3 °C, 6 °C and 9 °C	3
Ohsone et al. (1999)	Lettuce	5 °C, 10 °C, and 25 °C	3
Ringlé et al. (1991)	Broad leaf endive	4 °C and 8 °C	8
Steinbruegge et al. (1988)	Lettuce	5 °C, 12 °C and 25 °C	4
Szabo et al. (2003)	Iceberg lettuce	4 °C and 8 °C	2
Actia, unpublished data	Broad leaf endive	7 °C and 14 °C	7
Carlin, unpublished data	Lettuce, curly leaf endive, lamb's broad leaf endive	10 °C	7
Stahl, unpublished data	Lettuce	9 °C and 15 °C	6

and its uncertainty. This μ_{opt} value could be used for growth prediction in quantitative microbial risk assessment, for instance. The adequacy of the model was checked in comparing observed and predictive data, and additional data not used for deriving the posterior distributions of the model parameters.

2. Materials and methods

2.1. Data

Growth curves ($n=206$) showing the fate of *L. monocytogenes* in MP fresh leafy salads (lettuce including iceberg lettuce, broad leaf endive, curly leaf endive, lamb's lettuce, and mixtures of them) were selected from 21 peer-reviewed papers, and extended with growth curves from conference proceedings, technical reports, or unpublished laboratory data (Table 1). In most instances (time, *L. monocytogenes* $\log_{10}$ cfu/g) co-ordinates were taken from graphs. As in Pouillot et al. (2003), two *L. monocytogenes* strains were considered different if (i) they were different in one report or (ii) they were studied in different reports. In this way 43 modalities were defined.

2.2. Hierarchical Bayesian estimation of growth parameters

Let $i=1,\ldots,43$ denote the index of modality from $l_i=1,\ldots,L_i$ experimental growth curves. Each growth curve l_i is obtained at a temperature T_{l_i} (usually constant over the experiment) with J_{l_i} counts of *L. monocytogenes* noted $Y_{l_i}(t_j^{(l_i)})$ expressed in $\log_{10}$ cfu/g, where $(t_j^{(l_i)})$ is the incubation time. The observations $Y_{l_i}(t_j^{(l_i)})$ are modelled as in Pouillot et al. (2003) with the nonlinear model of Eq. (1):

$$Y_{l_i}\left(t_j^{(l_i)}\right)=f_{l_i}\left(t_j^{(l_i)}\right)+\varepsilon_{Y,l_i} \tag{1}$$

where $(\varepsilon_{Y,l_i})_{l_i=1,\ldots,L_i}$ for $i=1,\ldots,43$ are the independently and identically distributed (i.i.d.) random errors with a normal distribution of mean 0 and variance σ_Y^2, and $f_{l_i}(t_j^{(l_i)})$ is the function of the primary growth model given by the logistic equation with delay (Rosso et al., 1996):

$$f_{l_i}\left(t_j^{(l_i)}\right)=\begin{cases} Y_{0,l_i} & t_j^{(l_i)}\leq\lambda_{l_i}\left(T_{l_i}\right)\\ Y_{\max,l_i}-log_{10}\left(1+\left(10^{\left(Y_{\max,l_i}-Y_{0,l_i}\right)}-1\right)exp\left(-\mu_{\max,l_i}\left(T_{l_i}\right)\left(t_j^{(l_i)}-\lambda_{l_i}\left(T_{l_i}\right)\right)\right)\right) & t_j^{(l_i)}>\lambda_{l_i}\left(T_{l_i}\right)\end{cases}. \tag{2}$$

The parameters Y_0, $f(t)$ and $Y_{\max}$ are respectively the initial bacterial concentration, the bacterial concentration at time t and the maximal bacterial concentration in the product (unit: $\log_{10}$ cfu/g), $\lambda(T)$ is the lag time (h) at temperature T (°C), and $\mu_{\max}(T)$ (h^{-1}) is the maximal specific growth rate at temperature T. The initial concentration Y_0 and the maximal concentration $Y_{\max}$ are assumed to be curve-dependent, and their respective distributions are given in Table 2. As in Pouillot et al. (2003), the effect of the temperature on the maximal specific growth rate $\mu_{\max}$ is modelled with the nonlinear model of equation:

$$\sqrt{\mu_{\max,l_i}\left(T_{l_i}\right)}=\sqrt{g_{l_i}\left(T_{l_i}\right)}+\varepsilon_{\mu_{\max},l_i}, \tag{3}$$

where $(\varepsilon_{\mu_{\max},l_i})_{l_i=1,\ldots,L_i}$ for $i=1,\ldots,43$ are the i.i.d. random errors with a truncated normal distribution defined on $(0,+\infty)$, of mean 0 and variance $\sigma_{\mu_{\max}}^2$, and $g_{l_i}(T_{l_i})$ is the function of the secondary cardinal model (Rosso et al., 1995) written as follows:

$$g_{l_i}\left(T_{l_i}\right)=\begin{cases} 0 & T_{l_i}\notin\left[T_{\min,i},\ T_{\max,i}\right]\\ \dfrac{\mu_{opt,i}\left(T_{l_i}-T_{\max,i}\right)\left(T_{l_i}-T_{min,i}\right)^2}{\left(T_{opt,i}-T_{\min,i}\right)\left[\left(T_{opt,i}-T_{\min,i}\right)\left(T_{l_i}-T_{opt,i}\right)-\left(T_{opt,i}-T_{\max,i}\right)\left(T_{opt,i}+T_{\min,i}-2T_{l_i}\right)\right]} & T_{l_i}\in\left[T_{\min,i},T_{\max,i}\right]\end{cases} \tag{4}$$

where $T_{\min}$ and $T_{\max}$ are the theoretical minimal and maximal temperatures below and above which no growth occurs (unit: °C). T_{opt} is the theoretical optimal temperature at which $\mu_{\max}$ is highest and equal to the optimal growth rate μ_{opt} (h^{-1}), when the model

Table 2
Distributions used to model the growth parameters.

Growth parameters	Distributions[a]
$\mu_{opt,i}$	$N(M_{\mu_{opt}},S_{\mu_{opt}}^2)$
$T_{\min,i}$	$N(M_{T_{\min}},S_{T_{\min}}^2)$
$T_{opt,i}$	$N(M_{T_{opt}},S_{T_{opt}}^2)$
$T_{\max,i}$	$N(M_{T_{\max}},S_{T_{\max}}^2)$
$Y_{\max,l_I}$	$N(M_{Y_{\max}},\sigma_{Y_{\max}}^2)$
Y_{0,l_I}	$N(y_{0,l_I},\sigma_Y^2)I(0,+\infty)$
KL_{l_I}	$N(K_i,S_{KL}^2)I(0,\infty)$
K_i	$N(M_K,S_K^2)I(0,\infty)$

[a] $X\sim N(m,s^2)I(a,b)$, truncated normal distribution defined on the interval (a,b), of mean m and standard deviation s.