Quantitative microbial risk assessment for *Listeria monocytogenes* in cold smoked salmon

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Abstract

A wide variety of foodstuffs could be contaminated with *Listeria monocytogenes* (*Lm*), but in the majority of cases listeriosis is predominately related to ready-to-eat (RTE) food.

A stochastic model for the growth of *Lm* with the inhibiting effect of Lactic Acid bacteria (LAB) in cold smoked salmon (CSS) was developed. An existing model describing the inhibiting effect of LAB on the growth of *Lm* in CSS was extended using the Barany and Roberts model lag phase and stochastic models for growth rate and initial concentration. A deterministic model for the growth of *Lm* was adapted by adding the Winner stochastic process in order to simulate the growth of *Lm*.

The Poisson distribution is used to represent the initial count (occurrence) of *Lm*. A deterministic model for the growth of LAB is used and the inhibiting effects of *Lm* and LAB on each other are taken into account.

In order to estimate and predict the risk of illness from the stochastic mathematical models for growth of *Lm*, environmental conditions and dose response are used. The second order Monte Carlo (MC) simulation is used to obtain the probability density function (PDF) for the concentration of *Lm* at the moment of consumption of CSS. The Milstein algorithm has been used to solve the stochastic differential equation. The PDF for *Lm* obtained from the MC simulation is used in the dose response model to obtain the risk of illness at the moment of consumption. The Beta-Poisson model is used for the dose response.

Keywords: QMRA, cold smoked salmon, Listeria monocytogenes, stochastic model.

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1 Introduction

The food borne listeriosis is a relatively rare but serious disease with a very high rate of fatality (20-30%) compared to other food borne microbial pathogens, such as *Salmonella*. Foodstuffs are recognised as a primary route of transmission for human exposure [1–3].

A wide variety of foodstuffs could be contaminated with *Lm* but the majority of cases of listeriosis are predominately related to ready-to-eat (RTE) food. The important factor related to food borne listeriosis is that *Lm* can grow even under low temperatures (refrigerated temperatures) when given sufficient time. The RTE products with long shelf life are under risk with respect to the growth of *Lm* to critical concentrations [1, 4].

The CSS is a lightly preserved RTE product and control and prediction of the growth of *Lm* is of high importance in order to reduce the risk of listeriosis and to prevent recalls due to national and international regulations. For RTE food the European Union (EU) regulations differentiate between products that cannot and those that can support growth of *Lm*. According to the EU regulations, the maximal allowed concentration of *Lm* in the products that cannot support the growth of *Lm* is 100 CFU/g (EC 2073/2005) [5].

A Quantitative Microbial Risk Assessment (QMRA) model was developed for estimation of the risk to the consumers at the moment of consumption of the food. The risk is expressed through the probability for illness due to consumption of the product. The probability is obtained by using the probability density functions (PDF) for microbial concentration of the SFP obtained from a stochastic growth model and a dose response model for *Lm*. The high concentration of the LAB can inhibit growth of the *Lm* in lightly preserved seafood, through a phenomenon known as the Jameson effect [6, 7]. This effect was included in the growth model by coupling the growth equations for *Lm* and for LAB.

The stochastic fluctuations in the growth rate are taken into account by using white noise and the Winner process [8]. As the initial concentration and occurrence of the pathogens in different food packages in the supply chains are very low and rare, the Poisson distribution is used as the most appropriate mathematical model for this kind of stochastic process.

The stochastic growth equation for *Lm* is coupled with the deterministic one for the growth of LAB to include the inhibiting effects of LAB on the growth of *Lm* [9]. The Milstein algorithm was used to solve the stochastic differential equation numerically [10]. In the QMRA model the second order Monte Carlo simulation was used to simulate the stochastic process of microbial growth and random initial concentration of pathogenic microorganisms.

The gamma type of the secondary model, which includes different product parameters such as temperature [ºC], pH, water activity a_w, salt content NaCl%, smoke components (phenols), undissociated lactic acid LAC_U [mM/M], undissociated diacetate – DAC_U [mM/M], concentration of dissolved CO_2 [ppm] and growth/no growth boundaries, has been used to determine the growth rate of *Lm* [10–13].

2 The mathematical model for microbial growth

2.1 Primary model

As the high concentration of the LAB can inhibit growth of *Lm* in the lightly preserved seafood, such as CSS, through a phenomenon known as the Jameson effect, it is necessary to include this effect in the primary model for microbial growth [6, 7] of *Lm*.

As the initial concentration of the *Lm* is lower than concentration of the LAB, this effect will restrict maximal concentration of the *Lm*, which will have a great impact on the risk assessment in the RTE products. The growth rate of LAB in lightly preserved food products generally is lower than the growth rate that would inhibit growth of *Lm* [14]. The microbial interaction between *Lm* and LAB could be described by the following equations:

$$\begin{aligned}
&\frac{dN_{Lm}}{dt} = \alpha_{Lm}(t)\cdot \mu_{\max}^{Lm}\cdot\left(1-\frac{N_{Lm}}{N_{\max}^{Lm}}\right)\cdot\left(1-\frac{N_{LAB}}{N_{\max}^{LAB}}\right)\\
&\alpha_{Lm}(t) = \frac{q_{Lm}(t)}{1+q_{Lm}(t)};\quad \frac{dq_{Lm}}{dt} = \mu_{\max}^{Lm}\cdot q_{Lm}(t);\quad N_{Lm}(0) = N_{Lm0}\\
&\frac{dN_{LAB}}{dt} = \alpha_{LAB}(t)\cdot \mu_{\max}^{LAB}\cdot\left(1-\frac{N_{LAB}}{N_{\max}^{LAB}}\right)\cdot\left(1-\frac{N_{Lm}}{N_{\max}^{Lm}}\right)\\
&\alpha_{LAB}(t) = \frac{q_{LAB}(t)}{1+q_{LAB}(t)};\quad \frac{dq_{LAB}}{dt} = \mu_{\max}^{LAB}\cdot q_{LAB}(t);\quad N_{LAB}(0) = N_{LAB0}
\end{aligned} \tag{1}$$

where $\mu_{max}^{Lm/LAB}$ are maximal growth rate, $N_{max}^{Lm/LAB}$ are maximal concentrations without microbial interaction, and $N_{Lm/LAB0}$ are initial concentrations for *Lm* and LAB respectively, $q_{Lm/LAB}$ are quantities that are related to critical substance necessary for growth and characterize the physiological state of the cells at the moment of inoculation and $\alpha_{Lm/LAB}$ are adjustment functions for *L. monocytogenes* and LAB, respectively. The above model was extended using the Baranyi and Roberts model for the lag phase [9, 15]. The main assumption is that *L. monocytogenes* and LAB inhibit each other in the same extent [9]. The above equations do not have an analytical solution and could be solved numerically by discretization in time [15].

2.2 Secondary model

The growth rate as a function of the temperature [ºC], water activity a_w, pH, undissociated lactic acid LAC_U [mM/M], undissociated diacetate – DAC_U [mM/M], smoked components – phenols P [ppm], concentration of dissolved CO_2 [ppm] and nitrite NIT [ppm], could be expressed by the following equation [17]:

$$\mu_{Lm} = b \cdot \left(\frac{T - T_{\min}}{T_0 - T_{\min}}\right)^2 \cdot \frac{a_w - a_{w\min}}{a_{wopt} - a_{w\min}} \cdot \left(1 - 10^{pH_{\max} - pH}\right) \cdot \left(1 - \frac{LAC_U}{MIC_{lac}}\right) \cdot \left(1 - \sqrt{\frac{DAC_U}{MIC_{dac}}}\right) \cdot \left(\frac{P_{\max} - P}{P_{\max}}\right) \cdot \left(\frac{CO_{2\max} - CO_{2eq}}{CO_{2\max}}\right) \cdot \left(\frac{NIT_{\max} - NIT}{NIT_{\max}}\right)^2 \cdot \zeta \quad (2)$$

The MIC_{lac} and MIC_{dac} are theoretical concentrations of undissociated lactate and diacetate, respectively, preventing the growth of *Lm*. The pH_{max}, P_{max}, CO_{2max} and NIT_{max}, are maximal values for pH, phenols, concentration of the CO_2, and nitrite, respectively, which prevent the growth of *Lm*. The a_{wmin} and a_{wopt} are minimal and there is optimal water activity for the growth of *Lm*.

The concentration of the undissociated organic acid could be expressed using the following equation [17]:

$$LAC_U \Big/ DAC_U = \frac{TC}{1 + 10^{pH - pK_a}} \quad (3)$$

where TC [ppm] is the total concentration of the acid and the constant pK_a has values 3.86 and 4.76 for lactic acid and diacetate, respectively [17].

The concentration of dissolved CO_2 [ppm] could be calculated using Henry's low.

If the packaging is vacuumed the partial pressure is very low and could be considered as zero and dissolved concentration of the CO_2 could be neglected. The additional term in the equation (2), ζ, has value between 0 and 1 and it is added to describe the inhibition effect of the interaction between all product and environmental parameters [17].

2.3 Stochastic model for growth rate and initial concentration

The deterministic model for simultaneous growth of *Lm* and LAB gives only one single growth curve for both species which represents average value for microbial concentration. This type of model is not sufficient for microbial risk assessment and the stochastic component of the growth of *Lm* must be taken into account in order to estimate the potential risk to human health. The stochastic model should be used for prediction of the probability distribution of the *Lm* concentration at the moment of consumption [16]. On the other hand the model for growth of LAB should not be necessarily stochastic. A deterministic growth model would also take into account the inhibiting effect of LAB on the growth of *Lm* in average. The specific growth rate includes the deterministic part which is modelled using the secondary model presented by (2) and the stochastic part which is described by the white noise.

In this way all the probabilistic fluctuations due to the biological variability and uncertainty in the environmental parameters are taken into account. In this model the overall fluctuations are considered without separation between the uncertainty and variability. The specific growth rate is separated into deterministic and stochastic part using the following relation [8, 16]:

$$\mu_{stoc}^{Lm} = \mu_{\det}^{Lm} + \sigma_{Lm}(T) \cdot \xi_{Lm}(t) \tag{4}$$

where: ξ_{Lm} is stochastic processes described as white noise, σ_{Lm} is a model parameter related to the amplitude of the noise, μ_{det}^{Lm} is deterministic part of the growth rate and μ_{stoc}^{Lm} is the overall growth rate including deterministic and stochastic part [8, 16]. The connection between white noise and Winner process is given by the following equation:

$$dW(t) = \xi(t) \cdot dt \tag{5}$$

where W(t) is Winner process and ξ(t) white noise.

In general the occurrence of the *Lm* is very rear event due to different random processes such as cross-contamination or partitioning during the processes of packaging. Since the initial concentration of *Lm* is very low the initial microbial concentration can be modelled as a stochastic process, and the Poisson distribution would be used as a most suitable stochastic process for this kind of stochastic processes [16].

2.4 Dose response model

Once when probability distribution for microbial concentration is obtained using dose response the probability of illness at the moment of consumption could be obtained. The dose response model is a cumulative density function (CDF) which gives the probability for infection or to get ill if certain number cells are inoculated. In this study the Beta-Poisson model is used for dose response [20, 21]. In the Beta-Poisson model the average probability for infection is calculated assuming that the level of exposure follows the Poisson distribution. Using the mathematical model for dose response the probability for infection at the moment of consumption of food could be calculated using the following expression [22]:

$$P_{\inf} = \int P(M \cdot N) \cdot f(N) \cdot dN \tag{7}$$

where *M[g]* is average size of the portion, P is dose response or probability for infection for given number of cells, *N[CFU/g]* is microbial concentration and *f* is PDF for microbial concentration (for considered pathogen) at the moment of consumption.

3 Results

The results obtained by the developed numerical model are compared with laboratory data for growth of *Lm* and LAB in CSS. The numerical values for parameters used in the secondary model are shown in Table 1 [5, 9]. In Figure 1 the numerical results for microbial growth of *Lm* and LAB are compared with laboratory results. The growth rate for *Lm* is modelled as stochastic process and initial concentration was a deterministic quantity. The several stochastic growth

Table 1: The model parameters for *Lm* and LAB [5, 9].

Parameter	*Lm*	LAB
b	0.419	0.659
T_{min} [°C]	-2.83	-3.05
T_{ref} [°C]	25	25
a_{wmin} [%]	0.923	0.928
a_{wop} [%]	1	1
MIC_{LAC}[mM/M]	3.79	12
MIC_{DAC}[mM/M]	4.8	33.3
p_{max}[ppm]	28.1	40.3
CO_{2max}[%]	3.14	6.691
pH_{max}	4.97	4.24
pK_{aLAC}	3.86	3.86
pK_{aDAC}	4.76	4.76

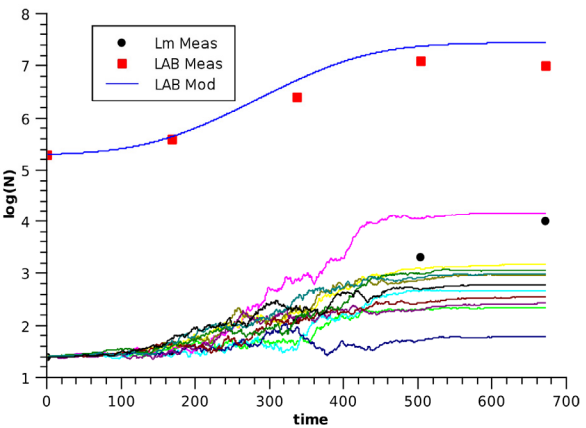

Figure 1: Comparison between laboratory results and prediction obtained using the mathematical model for growth of *Lm* and LAB. The stochastic growth curves for *Lm* are obtained by MC simulation. The initial count was considered as deterministic quantity. (Lm Meas = *Lm* laboratory results; LAB Meas = LAB laboratory results; LAB Mod = LAB modelling results).

curves for *Lm* obtained using MC simulation are shown. In Figure 2 the initial concentration for Lm was modelled as a Poisson process. The corresponding temperature profile is shown in Figure 3.

In Table 2 the probabilities to be ill after consumption of the food product are calculated. The MC simulation is performed for two different time intervals: 14 and 28 day and with and without Poisson distribution for initial count. When Poisson distribution is used two different mean values of the initial count are applied.

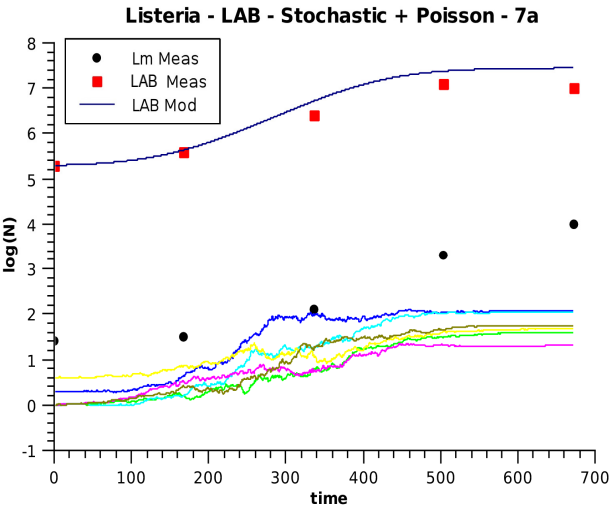

Figure 2: Comparison between laboratory results and prediction obtained using the mathematical model for growth of *Lm* and LAB. The stochastic growth curves for *Lm* are obtained by MC simulation. The initial count was modelled using Poisson distribution. (Lm Meas = *Lm* laboratory results; LAB Meas = LAB laboratory results; LAB Mod = LAB modelling results).

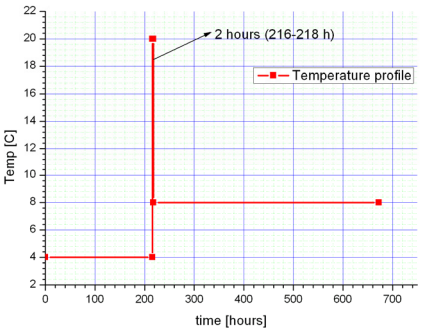

Figure 3: Temperature profile used in numerical simulation.

Table 2: Probability to be ill after 14 and 28 days. The simulation is performed with and without Poisson distribution for the initial count. When Poisson distribution is used a different mean value of initial count- N_{0mean} are applied.

	$\ln(N_{0mean})=1.38$	$\ln(N_{0mean})=3.22$	No Poisson
14 days	0.0381	0.1174	-
28 days	0.1078	0.2571	0.2573

4 Conclusions

The stochastic mathematical model for risk assessment in the CSS is developed. The Lm is considered as main pathogen and inhibiting effect of the LAB is included into the model. The coupled growth equation for *Lm* and LAB are considered. The stochastic nature of the growth for the Lm is modelled by separating the growth rate into deterministic and stochastic part. The deterministic part of the growth rate is modelled using secondary model. The secondary model includes different environmental parameters such as: temperature [ºC], water activity a_w, pH, undissociated lactic acid LAC_U [mM/M], undissociated diacetate – DAC_U [mM/M], smoked components – phenols P [ppm], concentration of dissolved CO_2 [ppm] and nitrite NIT [ppm].

For the stochastic part of the growth rate Winner process is used. The initial count of the *Lm* is modelled as stochastic quantity using Poisson distribution. The concentration of the LAB is considered as deterministic quantity. The system of two stochastic differential equations was solved using MC simulation and Milstein's algorithm. Using numerical simulation the PDF function for Lm concentration at different times is obtained. The Beta-Poisson distribution for dose response is used. Using the PDF and dose response model the risk to be ill after consumption of the food could be calculated. The results obtained by the numerical simulation are compared with laboratory data under dynamical temperature condition and good agreement is achieved.

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