Evaluation of QMRA performance for *Listeria monocytogenes* in cold smoked salmon

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Abstract

Food-borne listeriosis, caused by *Listeria monocytogenes (Lm)*, is relatively rare but the relatively high rate of fatality (20–30%) compared to other food-borne microbial pathogens such as *Salmonella* makes it a serious disease. The foodstuff is recognised as the primary route of transmission for human exposure. A wide variety of food or raw material may become contaminated with *Lm* but the majority of listeriosis cases are related to ready-to-eat (RTE) food. The important factor related to food-borne listeriosis is that *Lm* can grow under low (refrigerated) temperatures when given sufficient time. Therefore, RTE products with long shelf life are under risk with respect to growth of *Lm* to critical concentrations.

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 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 growth of LAB is used and the inhibiting effects of *Lm* and LAB on each other are taken into account. The Beta-Poisson model is used for estimating the dose response.

The model has been tested during field trials with CSS performed in August 2010. The salmon was slaughtered in Norway and transported to France where it was processed. The model, implemented within the QMRA module, indicated that growth of Lm would occur in the CSS samples investigated. However, the

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data obtained during the field trial showed that microbial cell counts implied a reduction in the population of Lm with storage time, which means that a different model to describe the growth of Lm in presence of LAB may be required, especially for lower concentrations of Lm.
Keywords: QMRA, Listeria monocytogenes, cold smoked salmon, model evaluation.

1 Introduction

Illness caused by *Listeria monocytogenes* (*Lm)* is more rare but of more concern because of the seriousness of the illness which can cause death particularly in pregnant women, young children, elderly and immuno-compromised people [1, 2]. *Lm* is widely spread in the environment because it is resistant to different environmental conditions and it can grow in soil, water, fodder, straw, feed stuffs or faeces. The bacterium is therefore present in a wide variety of raw food. It is able to grow at refrigeration temperatures and survive in food for longer periods under adverse conditions [3]. Its ability to colonise food processing environments has been reported [4]. *Listeria* is able to attach itself to working surfaces creating biofilms that are difficult to remove [5, 6], and become a contamination source in food processing [7]. The contamination of the product in cutting and chilling areas is mainly due to cross contamination.

For RTE food the European Union (EU) regulations differentiate between products which cannot and those which can support growth of *Lm*. According to the EU regulations, the maximal allowed concentration of *Lm* in the products which cannot support the growth of *Lm* is 100 CFU/g (EC 2073/2005) [8].

A Quantitative Microbial Risk Assessment (QMRA) model was developed for estimation of the risk to the consumers expressed through the probability for illness due to consumption of CSS. The probability is obtained by using the probability density functions (PDF) for microbial concentration of *Lm* obtained from a stochastic growth model and a dose response model for *Lm*. The stochastic fluctuations in the growth rate are taken into account by using white noise and the Winner process [9]. The Poisson distribution is used as the initial concentration and occurrence of the pathogens in different food packages in a supply chain, where these are very low and rare, respectively.

High concentrations of LAB can inhibit growth of the *Lm* in lightly preserved seafood, and therefore the stochastic growth equation for *Lm* is coupled with the deterministic model for the growth of LAB to include the inhibiting effects of LAB on growth of *Lm* [10]. The Milstein algorithm was used to solve the stochastic differential equation numerically [11]. 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 model includes the influence of various parameters on the growth of *Lm* and LAB, such as: temperature, pH, water activity (a_w), salt content (NaCl), smoke components (phenols), undissociated lactic acid LAC_U, undissociated

diacetate – DAC_U, and concentration of dissolved CO_2. More on the developed model can be found elsewhere [12, 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 lightly preserved seafood, such as CSS through the phenomenon known as Jameson effect, it is necessary to include this effect in the primary model for microbial growth of *Lm* [14, 15].

As the initial concentration of *Lm* is lower than the concentration of LAB this effect will restrict maximal concentration of *Lm* which will have great impact on the risk assessment of 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* [16]. 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 $N_{Lm0/LAB0}$ are initial concentrations for *Lm* and LAB, respectively; $q_{Lm/LAB}$ are quantities which are related to critical substance necessary for growth and characterise the physiological state of the cells at the moment of inoculation and $\alpha_{Lm/LAB}$ are adjustment functions for *Lm* and LAB, respectively. The above model was extended using Baranyi and Roberts model for taking into account the lag phase [10, 17]. The above equations do not have analytical solution and could be solved numerically by discretisation in time [17].

2.2 Secondary model

The growth rate as a function of the temperature, water activity (a_w), pH, undissociated lactic acid (LAC_U), undissociated diacetate (DAC_U), smoked components (phenols, P), concentration of dissolved CO_2, and nitrite (NIT), could be expressed by the following equation [18]:

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

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}, NIT_{max}, are maximal values for pH and concentration of phenols, CO_2, and nitrite, respectively, which prevent growth of *Lm*. The a_{wmin} and a_{wopt} are minimal and optimal water activity for growth of *Lm*.

2.3 Stochastic model for microbial growth 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 cannot be applied in microbial risk assessment and therefore a stochastic model for growth of *Lm* is used in this work [12]. On the other hand the model for growth of LAB was taken to be deterministic as it provides the inhibiting effect of LAB on the growth of *Lm*, for which the average value is sufficient, reducing this way the required central processor unit (CPU) time. The Poisson distribution was used as a most suitable for this kind of stochastic process [12].

2.4 Dose response model

Once the probability distribution for microbial concentration is obtained by using the dose response the probability for illness in consumers could be estimated. The dose response model is a cumulative density function (CDF) which gives the probability for infection or to get ill if a certain number of cells are inoculated. In this study the Beta-Poisson model is used for dose response [19, 20]. More on the developed model can be found elsewhere [12, 13].

2.5 Field trial experimental set-up and analysis of naturally Lm-contaminated CSS

Farmed salmon was slaughtered near Alesund in Norway (Aukra), and transported to Boulogne sur Mer, France, where it was processed into CSS fillets five days post-mortem. The sliced and vacuum-packaged product was ready two days later. The packages were assigned to 3 different treatments, aiming to study the effect of isothermal (3 and 10°C) or abusive temperature on the *Listeria* risk for these products. The abusive treatment implied shipping few packages to Paris during which high thermal load was applied twice (>20°C product temperature), followed by a low (3°C) temperature storage. Temperature of two packages was monitored every 10-min using temperature data loggers for each treatment investigated. The storage trial lasted for 27 days during which Lm and LAB analyses were performed regularly by a Cofrac-accredited French laboratory. Detection in 25 g (BRD-07/4-09/98) and enumeration (RLM-V9-13/10/06|BRD-

07/05-09/01) of Lm was conducted on duplicate samples for each treatment. Nitrite-Actidione-Polymyxin (NAP) agar [21] was used for LAB enumeration following incubation at 25°C for 3–5 days and confirmation by testing for the presence of catalase-negative colonies.

3 Results

The QMRA model for CSS was tested during the cold smoked salmon field trial in August 2010. The salmon was slaughtered near Alesund in Norway (Aukra), and delivered to Boulogne sur Mer, France, 5 days later where it was processed, sliced and vacuum-packaged by a processor. CSS products were stored at two temperatures (3 and 10°C) and a third treatment considered shipment of few samples to Paris, allowing a temperature abuse of few hours and brought back to laboratory storage at 3°C. The presence (detection and enumeration) of *L. monocytogenes* in the cold smoked salmon was regularly verified throughout the trial (27 days) and found to be naturally occurring from early post-packaging (25 h). The three different temperature profiles used in the study are shown in Figure 1, labelled as *Treatment 1*, *Treatment 2* and *Paris*. The laboratory results of microbial growth show that LAB reached high levels after 19 days of storage, being higher at 10°C than 3°C.

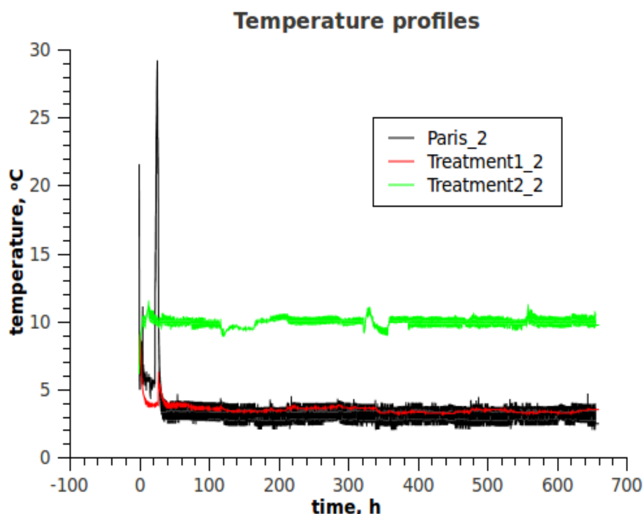

Figure 1: Temperature profiles applied to CSS products.

The experimental counts of the microbial population for Treatments 1 and 2 are shown in Tables 1 and 2, respectively. The presence of *Lm* was confirmed 25 h post-packaging and up to day 19. However, one of the samples was negative after 19 days of storage while all samples were negative on day 27. Up to day 19, the suspected *Lm* level ranged between 0.04 to just below 10 cells/g

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while it had decreased to less than 0.04 cell per g on day 27. This demonstrates the very low *Lm* level in the product and even its sporadic incidence in the whole batch. It is also likely that the inhibitory activity of LAB may have contributed to the *Lm* growth control and, ultimately, the bactericidal effect observed towards the end of the storage life.

These results are not in agreement with the developed QMRA model. The model does not take into account reduction in number of colony-forming units (CFU) and therefore cannot predict the disappearance of *Lm* towards the end of the field trial.

Table 1: Laboratory results for the microbial population counts (log N CFU/g) of Treatment 1 group (3.5 ± 0.4°C) in duplicate samples (S1 and S2).

Time (h)	LAB counts		Lm detection*	
	S1	S2	S1	S2
25.02	3.90	2.65	+	+
119.75	3.67	3.40	+	+
290	4.30	4.84	+	+
462	5.11	5.56	-	+
658	5.64	4.18	-	-

*Detection in 25 g sample. Enumeration conducted always gave < 10 CFU/g.

Table 2: Laboratory results for the microbial population counts (log N CFU/g) of Treatment 2 group (9.9 ± 0.4 °C) in duplicate samples (S1 and S2).

Time (h)	LAB counts		Lm detection*	
	S1	S2	S1	S2
25.02	3.90	2.65	+	+
119.75	3.52	5.15	+	+
290	>7.48	4.49	+	+
462	6.38	>8.48	+	+
658	8.15	8.48	-	-

*Detection in 25 g sample. Enumeration conducted always gave < 10 CFU/g.

Based on initial mean LAB load (log (N)=3.3 CFU/g), *Lm* and LAB growth were calculated under all three temperature profiles obtained (T1, T2, and P) assuming Lm counts of 0.04 cell/g and 10 cells/g. The lower level is based on the fact that detection of *Lm* in 25 g implies the presence of at least one living cell, hence 0.04 cell/g.

Figures 2 and 3 show the comparison between the laboratory counts for LAB and the corresponding model predictions for growth of LAB and *Lm* for initial

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Lm count of 0.04 CFU/g for Treatment 1 and Treatment 2, respectively. The model performed calibration during the calculation of bacterial growth.

The results in Figure 2 show slow growth, however, the microbiological results suggest that *Lm* concentration is declining with storage time, perhaps due to interaction with LAB. At higher storage temperature, Figure 3 shows faster

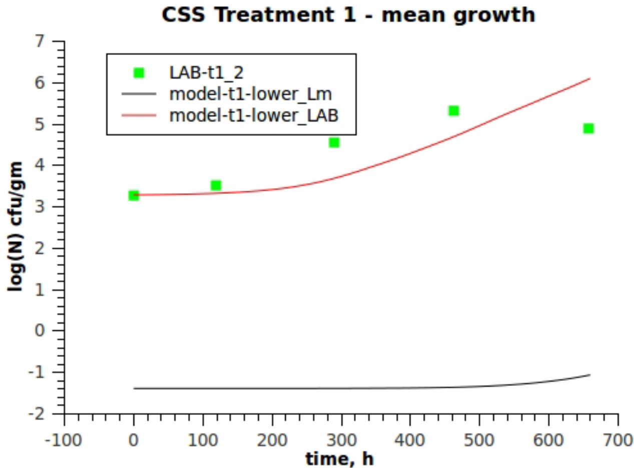

Figure 2: Comparison between observed counts of LAB (lactic acid bacteria) and predicted LAB growth along with the growth prediction for *Lm* (*L. monocytogenes*) for Treatment 1 profile. "lower" in the legend refers to 0.04 CFU/g.

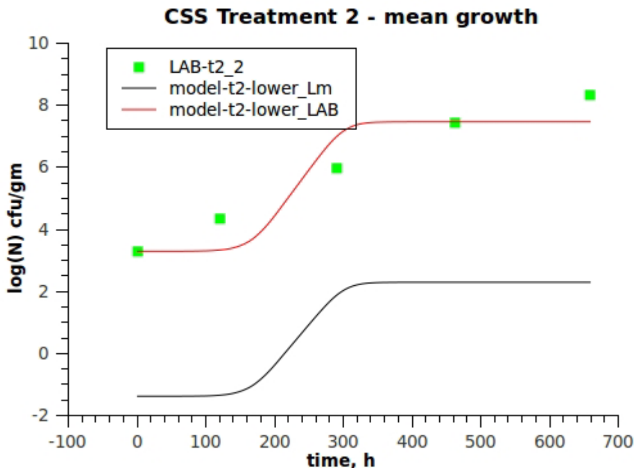

Figure 3: Comparison between observed counts of LAB (lactic acid bacteria) and predicted LAB growth along with the growth prediction for *Lm* (*L. monocytogenes*) for Treatment 2 profile and initial *Lm* count of 0.04 CFU/g.

Lm growth, in contrast to the data observed. The main conclusion is that the model reported by Mejlholm and Dalgaard [10], which has been modified to include automatic calibration for the lag phase and also stochastic element for growth and initial count, may not be applicable for low concentrations of *Lm*. The microbiological results show that the interaction with LAB reduces *Lm* concentration below the detection limit, while the model shows growth once the lag phase is finished and may stop the growth if LAB or Lm counts reach the maximum cell level, but cannot reduce the cell count under any conditions. This is not a characteristic of the dataset used to prepare the model. In fact, this is the characteristic of the model equations which do not allow for decrease in *Lm* concentration.

The QMRA model provides the risk to consumers due to consumption of the product. Table 3 shows the QMRA results for the three temperature profiles and two different initial *Lm* concentrations, 0.04 and 10 CFU/g. The calculated probability for illness in consumers for low initial concentration of *Lm* and lower average temperature results in a very low incidence, which is of order 2 to 3 cases of illness per 100 million portions, in this case each taken as 100 g. For the case of lower initial concentration and higher average temperature the probability for illness rises to 4,400 cases of illness per 100 million portions. This probability is of three orders of magnitude higher than for the case of lower average temperature, and one can argue that it might be high enough to allow establishing a link between the illness and the product. Due to the variability in response in humans to *Lm* it is still unlikely under such low probability to have two cases of illness in the same household or between the affected person and the close contacts, which is usually the easiest way of establishing link between food source and illness. Such low probability might not cause sufficient incidence of illness in an area to establish an outbreak, therefore the source may not be detected.

Under higher initial concentration and lower average temperature the probability for illness increases to approximately 10,000 cases of illness per 100 million portions. This is approximately twice as high probability as the one for lower initial concentration of *Lm* and higher average temperature. However, the above discussion on likeliness of establishing a link between illness and food source would still apply.

Table 3: Risk of illness in consumers, as influenced by *Lm* initial level and temperature conditions, according to the stochastic QMRA model.

Profile (**Mean product temp.**)		**Treatment 1** (3.5 ± 0.4 °C)	**Treatment 2** (9.9 ± 0.4 °C)	**Paris** (3.4 ± 1.0 °C)
Risk of illness	0.04 CFU/g	0.000002%	0.0044%	0.000003%
	10 CFU/g	0.0096%	0.5639%	0.0127%

For the case of higher initial concentration of *Lm* and higher average temperature the probability for illness rises to 5.6 cases of illness per 1 thousand portions (or 0.56% of a population). This incidence is high enough to establish a link between the food product and the illnesses in consumers which might result in an outbreak.

4 Conclusions

The model of Mejlholm and Dalgaard [10], implemented within the QMRA module, indicated that growth of *Lm* would occur in the investigated CSS samples. The field trial microbial cell counts implied a reduction in the population of *Lm*, which means that a different model to describe the growth of *Lm* in presence of LAB may be required, especially for lower concentrations of *Lm*. The inaccuracy in the predictions cannot be helped by the automatic calibration of the model [13] for two reasons: (i) the concentrations are on the detection limit and therefore the exact cell counts cannot be determined, which provides only information on whether *Lm* is detected or not detected; (ii) it is inherent property of the model not to be able to predict decrease of the microbial population of *Lm*.

To incorporate the reduction in cell counts of *Lm* in the model, in order to reflect the observed microbial counts, a model for the *Lm* growth in CSS in presence of LAB, which is based on the Lotka-Volterra type of equations can be used, as such model can predict both conditions; growth and decline in cell numbers of *Lm*.

Such model would require substantial amount of structured laboratory experiments in order to determine the model parameters. However, these results are indicative of the need for new models in order to describe the interaction of LAB with *Lm* at low contamination level.

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