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IMPACT OF COOKED HAM COLD CHAIN VARIABILITY ON SAFETY BY SENSITIVITY ANALYSIS

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ABSTRACT

Deterministic models describing heat transfer in cold chain, microbial growth and product quality evolution are widely studied. However, it is difficult to apply them in practice because of several random parameters of the logistic supply chain (ambient temperature varying due to season, product residence time in equipment...), and of the product characteristics (initial microbial load, lag time, water activity...). This variability can lead to different product evolutions (microbial load, weight losses, firmness and colour change) causing product losses and health risks.

This study proposes a new approach combining the deterministic and stochastic modelling (Monte Carlo) to take into account the variability of the logistic supply chain and product characteristics. The developed methodology was applied to the cold chain of cooked ham including, display cabinet, transport by consumer and domestic refrigerator, to predict the evolution of state variables such as temperature and the growth of *Listeria monocytogenes*. The impact of inputs parameters was calculated and ranked to highlight the main sources of product degradation.

Keywords: heat transfer, chilled food, variability, *Listeria monocytogenes*

1. INTRODUCTION

Temperature control along the cold chain is essential to maintain product quality and thus reduce food losses and health risks. Each year, in Europe *Listeria monocytogenes* is involved in more than one thousand cases of listeriosis (EFSA, 2012) with a fatality rate of about 20% among reported cases. These cases of listeriosis are mainly related to ready-to-eat dairy, vegetables and meat products. This pathogen is problematic for food industry because it is widespread in the environment and able to grow in the temperatures of the chilled products and also in a wide range of product properties (pH, water activity, CO₂). In European Union regulation, food safety criteria are proposed to “define the acceptability of a product or a batch of foodstuff applicable to products placed on the market.” The food safety criteria for RTE foods that are able to support the growth of *L. monocytogenes*, is based on compliance with the criteria of absence of the pathogen (in 25 g) before the food has left the immediate control of the Food Business Operator (FBO) and levels up to 100 CFU/g in products placed on the market during their shelf-life (Anonymous, 2005).

In this context, predictive microbiology and Quantitative Microbiology Risk Assessment (QMRA) have become important tools of food safety as they help FBO and competent authorities to define shelf-life or the impact of control measure on risk level. However, users of both predictive microbiology and QMRA generally consider simple time / temperature histories for assessing *L. monocytogenes* levels throughout the shelf life. Variability observed in the supply chain is not taken into account (ambient temperature varying due to season, product position and residence time in equipment). A few study such as those of Afchain et al.(2005) and Pouillot et al. (2007), based a French survey (ANIA, 2004) implemented the variability of the time / temperature history along the cold chain of cold cooked salmon in a QMRA to predict the exposure of *L. monocytogenes* but a constant product temperature was assumed in equipments.

This study proposes a new approach combining the deterministic and stochastic modelling (Monte Carlo) to take into account the variability of the logistic chain and product characteristics. The developed

methodology was applied to sliced cooked ham including the last three links of the cold chain: display cabinet, transport by consumer and domestic refrigerator. The evolution of state variables such as temperature and microbial load was predicted. To reduce the complexity of these models due to the high number of input parameters, Augustin (2011) proposes to identify the relevant parameters by means of Sensitivity Analysis (SA). SA is defined as the study of the output parameter uncertainty related to different sources of uncertainty in the input parameters (Saltelli et al., 2004). Among several methods of SA (Scatter plot, ANOVA, regression analysis), variance based methods seem to be adapted to non-linear models like those in QMRA and are recognized in the statistical field (Ellouze et al., 2010).

The aim of this study is to combine a heat transfer model of equipments (Flick et al., 2012; Hoang et al., 2012) and a microbiological model to predict the contamination of *L. monocytogenes* in cooked ham at the consumption point. A sensitivity analysis was performed to highlight and rank the parameters which have the higher impact on the contamination level at consumption point.

2. MATERIALS AND METHODS

An overview of the methodology, the model development and the Sensitivity Analysis (SA) are presented below.

2.1. Overview of the methodology

The global methodology is presented in the Figure 1, three parts can be distinguished. It should be noted that only contaminated packages were considered. Contamination level were predicted from the display cabinet in supermarket (time= t_0) to the consumption point (time= t_{end}). The part 1 presents the initial biological parameters (initial contamination y_0 , lag time represented by physiological state Q_0 of the cells that contaminate the products), product properties (pH, Aw, NIT and CO₂) and time / temperature profile of I packages. The part 2 illustrates the relationship between the primary growth model which aims to compute the bacterial growth with time $y(t)$ (log₁₀cfu/g) as a function of the initial contamination, physiological state and secondary growth model which allows taking into account the environmental parameters (properties and time / temperature histories of products). The growth and the impact of competitive flora are not taken into account in this study. The output parameter of the part 2, final contamination y_{end} , will be used as input in the SA (part 3) to assess the impact of inputs parameters. The detail of model development is presented in 2.2.

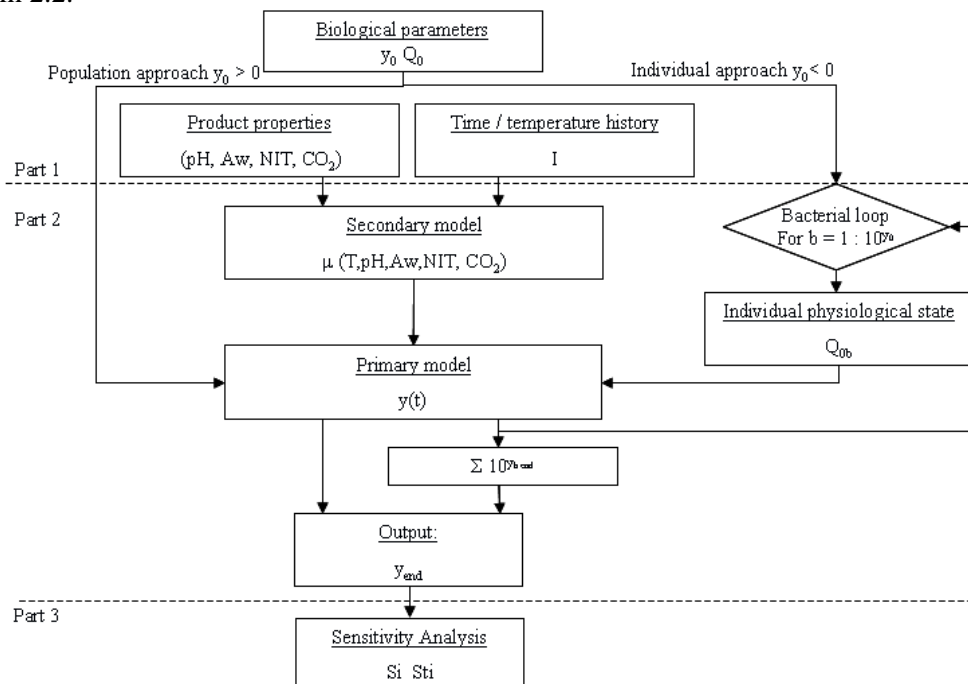


Figure 1 : Organigram to estimate the sensibility of input parameters on the final concentration of *L. monocytogenes*

2.2 Model development

2.2.1 Modelling the time / temperature history

Time / temperature history of the product along the cold chain was modelled using the methodology developed by Flick et al.(2012). This study proposes a general methodology combining determinist models for equipment and product evolution and stochastic models to consider different sources of variability of the cold chain (sequence of equipments, ambient conditions and thermostat setting temperature). The variability of these parameters is characterized by a Probability Density Functions (PDF). The studied product, called 'product of interest', can follow different itineraries (type of equipment, position and residence time). This methodology was applied by Hoang et al. (2012) to the pre-package meat cold chain (without water transfer).

The survey data in France (ANIA, 2004) were used to estimate the residence time of ham in the different links of the cold chain.

It is to be emphasised that the temperature $T(t)$ of the product of interest changes with time and it approaches the load temperature $T_{load,j,l}$ (other product located near the product of interest). The load temperature is assumed to be time independent and can be calculated from the equipment parameters (ambient temperature, thermostat setting temperature, etc) using equations developed by Laguerre et al (2010a) for display cabinet and Laguerre and Flick (2010b) for domestic refrigerator. In these studies, 4 load positions are considered in a display cabinet (top/front, top/rear, bottom/front and bottom rear) and 2 positions in domestic refrigerator (top and bottom). This methodology is applied to each link j of product number i at the position l in the equipment. The temperature evolution is calculated using the following equation:

$$T_{i,j,l}(t) = T_{0,i,j,l} + (T_{load,j,l} - T_{0,i,j,l}) \exp \frac{H_{j,l} t}{mC} \quad (1)$$

where $T_{i,j,l}(t)$ is the temperature of the product of interest i in the link j and position l , $T_{0,i,j,l}$ is the initial temperature of the product of interest i in the link j and position l , $T_{load,j,l}$ is the load temperature in the link j and position l . For ham, the weight m and the thermal capacity C are assumed to be constant and equal to $m = 160$ g and $C = 3500$ J.kg⁻¹.°C⁻¹, respectively. The conductance $H_{j,l}$ was measured for each position l of each link j .

More details about the development of this methodology have been presented in Flick et al (2012) and Hoang et al (2012).

2.2.2 Modelling the initial contamination

The initial contamination of *L. monocytogenes*, y_0 , in products was assessed from a survey undertaken from January 1997 to December 1998 in a big supermarket in Belgium (Uyttendaele et al., 1999). Data of cooked ham after slicing were used, among 879 products, *L. monocytogenes* was detected in 54 products (sample of 25 g) and 8 of them were detected in sample of 0.1g. The distribution of the initial contamination was evaluated using the *fitdistrplus* package of the R software (Pouillot and Delignette-Muller, 2010). This package includes a set of functions dedicated to facilitating the entire process of fitting parametric distributions for different types of data, including censored data.

2.2.3 Modelling the growth of *Listeria monocytogenes*

Growth of *L. monocytogenes* in cooked ham was predicted from the display cabinet to the consumption. The output parameter of the model is the bacterial contamination at the consumption point y_{end} (log₁₀cfu/g). Two approaches are mainly used to evaluate the initial physiological state of microorganism Q_0 : populational and individual approaches. The first one considers the global growth of the population and assigns one value to the whole population. The variability of Q_0 is deduced from the experimental data of Guillier and Augustin (2006). In this study, *L. monocytogenes* were stressed by benzalkonium chloride (BAC) which is disinfectant largely used in food industry. The second one (individual approach) considers a different physiological state $Q_{0,b}$ for each cell of a population. Individual physiological states $Q_{0,b}$ are deduced from a different expression of the individual physiological state, h_{0b} given by eq. (2).

$$Q_{0b} = \ln(1/(\exp(h_{0b}) - 1)) \quad (2)$$

Initial physiological state h_{0b} are calculated from the population initial physiological state h_0 using an Extreme Value type II distribution (EVII) characterised by 3 coefficients: a , b and c :

$$b = D/0.3658, a = M - 1.1645 \cdot b \text{ and } c = 5 \quad (3)$$

with M and D, the mean and the standard deviation of individual cell physiological state, respectively calculated with the following equation (Guillier and Augustin, 2006; Guillier and Augustin, 2008):

$$D = \exp(1.004 \cdot \ln(M) - 0.447) \quad (4)$$

$$M = \exp(0.0103 \cdot (\ln(h_0))^5 + 0.0065 \cdot (\ln(h_0))^4 - 0.039 \cdot (\ln(h_0))^3 + 0.0586 \cdot (\ln(h_0))^2 + 1.1941 \cdot \ln(h_0) + 0.1549) \quad (5)$$

The growth of each cell was calculated independently. The final contamination is the sum of sub-population of each individual cell of the initial contamination. It was assumed a limit value of 10 cells between the populational and individual approaches.

The primary model is an exponential growth model with lag time proposed by Baranyi and Roberts (1994):

$$\frac{dy(t)}{dt} = \frac{1}{1 + e^{-Q(t)}} \times \mu_{\max}(T, E) \times (1 - e^{y(t) - y_{\max}}) \quad (6a)$$

$$\frac{dQ(t)}{dt} = \mu_{\max}(T, E) \quad (6b)$$

$$y(0) = y_0 \quad Q(0) = Q_0 \quad (6c)$$

where $y(t)$ is the bacterial load ($\log_{10}\text{cfu/g}$) at the time t , y_0 the initial bacterial concentration, y_{\max} the maximum bacterial concentration, Q_0 the initial physiological state, μ_{\max} the maximum specific growth rate (h^{-1}), T the product of interest temperature ($^{\circ}\text{C}$) and E the product characteristics (A_w , pH, NIT, CO_2). To predict the microbial growth under dynamic conditions and describe the effect of environmental conditions (temperature, product properties), a simplified expression of the secondary model proposed by Mejlholm et al (2010) was used:

$$\mu_{\max} = \mu_{\text{ref}} \left[\frac{(T - T_{\min})}{(T_{\text{ref}} - T_{\min})} \right]^2 \times E \quad (7a)$$

with :

$$E = \left(\frac{A_w - A_{w_{\min}}}{A_{w_{\text{opt}}} - A_{w_{\min}}} \right) \times \left[1 - 10^{(pH_{\min} - pH)} \right] \times \left(\frac{\text{NIT}_{\max} - \text{NIT}}{\text{NIT}_{\max}} \right) \times \left(\frac{\text{CO}_{2\max} - \text{CO}_{2\text{equilibrium}}}{\text{CO}_{2\max}} \right) \quad (7b)$$

where μ_{ref} is the reference specific growth rate with a value of 0.419 h^{-1} for μ_{\max} at the reference temperature (T_{ref}) of 25°C , A_w is the water activity, NIT and $\text{CO}_{2\text{equilibrium}}$ are the concentrations (ppm) of nitrite, and dissolved CO_2 at equilibrium, respectively. T_{\min} , $A_{w_{\min}}$, pH_{\min} , NIT_{\max} and $\text{CO}_{2\max}$ are the theoretical minimal value of temperature, water activity, pH and maximal concentrations (ppm) of nitrite and CO_2 , respectively, that allow growth of *L. monocytogenes*. The effect of interactions between environment parameters was not considered. Product temperature is a time dependant parameter while product properties (pH, A_w , CO_2 , NIT) are time independent. However, the variability of product properties is taken into account using a survey data of 50 ham products of 10 different brands (Ifip, 2010).

The Runge Kutta method (ODE 45 function of Matlab software vR2012a, The MathWorks Inc., Natick, MA, USA) was used to solve the differential equation of the primary model.

2.3 Sensitivity Analysis

To perform sensitivity analysis, the Saltelli method (Saltelli, 2002) was chosen. This method was recommended by Augustin (2011) in QMRA because of its simplicity of implementation and acceptable computation time. SA is based on three steps: (i) definition of range of variation, (ii) model running (iii) computation of first order (S_i) and total effect (S_{ti}) indices. Parameters of the model were estimated from several sources: surveys, experiment and literature. A range was considered for inputs parameters to perform SA except for itinerary I of the products along the cold chain.

2.3.1 Range of variation of input parameters

The range of variation of the input parameters is presented in the Table 1. The range of the initial contamination y_0 is the 1st and 99th percentile of the normal distribution provided by Uyttendaele (1999). The initial physiological state Q_0 was obtained with experimental data, taking the value of Q_0 for the stress with BAC (-2.92) for the lower boundary and considering that there was no lag time for the upper boundary ($Q_0=8.29$ for a lag time of 1s in the experimental conditions of Guillier and Augustin (2006)). Product

property boundaries E correspond to the maximal and minimal values of the survey of Ifip (2010). μ_{ref} and T_{min} ranges were obtained from Augustin (2005) and (Mejlholm et al., 2010), respectively. Finally, the impact of cold chain itinerary for 10000 packages was considered.

Table 1 : Input parameters and the range of variations

Factor	Unit	Range	References
y_0 (initial contamination)	$\log_{10}\text{CFU g}^{-1}$	0 / 3,05	Uyttendaele et al (1999)
Q_0 (initial physiological state)	dimensionless	- 2,92 / 8,29	Guillier and Augustin (2006)
E (product characteristics)	dimensionless	0,396 / 0,642	Ifip (2010)
μ_{ref} (reference growth rate)	h^{-1}	0.2 / 0.6	Augustin (2005)
T_{min} (minimum growth temperature)	$^{\circ}\text{C}$	-3,99 / -1,19	Pouillot et al (2003)
I (product itinerary)	dimensionless	10000	-

2.3.2 Computation of Saltelli's indices

Saltelli's SA is based on a numerical procedure for computing the full set of first-order indices, S_i , and total effect indices, St_i , for all the studied factors k ($k = 1, \dots, K$). Two matrices A (N, K), B (N, K) (N number of simulation runs, K factors) were generated from a space filling method, the Latin Hypercube Sampling (LHS) (Helton and Davis, 2003) with respect to the range of variation of each factor. Then, k matrices C_k ($k = 1, \dots, K$) identical to B but with the k^{th} column replaced by the k^{th} column of the matrix A were built. For each row of the $k + 2$ matrices, the model was run and provided $k + 2$ vectors (Y_A, Y_B, Y_{C_i}) filled with values of the final concentration of the product y_{end} . First order indices S_i and total effect indices St_i were computed using the following expressions:

$$S_i = \frac{\frac{1}{N} \sum_{u=1}^N Y_A^{(u)} Y_{C_i}^{(u)} - g_0}{\frac{1}{N} \sum_{u=1}^N Y_A^{(u)} Y_A^{(u)} - f_0^2} \quad (8)$$

$$St_i = \frac{\frac{1}{N} \sum_{u=1}^N Y_B^{(u)} Y_{C_i}^{(u)} - f_0^2}{\frac{1}{N} \sum_{u=1}^N Y_A^{(u)} Y_A^{(u)} - f_0^2} \quad (9)$$

$$\begin{aligned} f_0 &= \frac{1}{N} \sum_{u=1}^N Y_A^{(u)} \\ g_0 &= \frac{1}{N} \sum_{u=1}^N Y_A^{(u)} Y_B^{(u)} \end{aligned} \quad (10)$$

3. RESULTS AND DISCUSSION

Input parameters were ranked according to their total effect indices obtained with 10^4 runs of LHS indices and presented in the Table 2. Differences observed between first order (S_i) and total effect (St_i) indices illustrate that the output variance was also due to interactions between input parameters and justify the use of variance based sensitivity analysis. Moreover, the sum of first order indices (0.91) indicates that the contribution of the individual effect of input parameters explained 91% of the total output variance, interactions between input parameters represents 9% of the total output variance.

Table 2 : Influence of studied factors on the final contamination at the consumption (classified by the order of importance), estimated by the individual effect (S_i) and Total Effect (St_i)

Factor	S_i	St_i
I (itinerary)	0.52	0.61
y_0 (initial contamination)	0.28	0.35
μ_{ref} (reference growth rate)	0.06	0.14
E (product characteristics)	0.02	0.11
T_{min} (minimum growth temperature)	0.01	0.09
Q_0 (initial physiological state)	0.02	0.08

In our case, the most important factor influencing the variance of the contamination at the consumption point was the itinerary of the product along the cold chain (I). The itinerary was previously identified as one of the most important factor in an exposure assessment model of *L. monocytogenes* in cold smoked salmon (Ellouze et al., 2010; Pouillot et al., 2007). This result shows on one hand the importance of food retailer practices and the consumer behaviour and on other hand that a special attention has to be paid in QMRA regarding time / temperature profiles. The second important parameter was the number of initial contaminating cells (y_0) previously identified by Pouillot et al (2007) but less significant in Ellouze et al (2010) and Augustin (2011). Differences between the results of these studies highlight the importance of the range of variation of input parameters using Saltelli method. The high impact of initial contamination in our study can also be explained by the number of input parameters. In fact, Saltelli indices are relative and the number of input parameters can explain the significance or not of the initial contamination in the different studies. The reference growth rate, the product characteristics, the minimum temperature of growth and the initial physiological state have a slight impact on the final contamination. The value of these parameters can be fixed at their mean value in order to simplify the model and thus, decrease the calculation time without affecting the accuracy of the prediction.

The developed methodology enables the quantification of the impact of the whole cold chain logistic on food safety. However, it does not highlight the parameter which has the greater influence because of the variability of equipments and positions. Saltelli's indices allow simplifying the model and develop a more detailed sensitivity analysis focused on the last three links of the cold chain. It is possible to assess the impact of residence time, load temperature and also to compare different positions in equipment by means of accept and reject algorithm (Guillier et al., 2011). These aspects, however, are not the subject of this preliminary study.

4. CONCLUSION

A methodology combining deterministic and stochastic models applied to cook ham was developed. The models allow the prediction of the *L. monocytogenes* contamination in the display cabinet, transport by consumer after purchase and domestic refrigerator. The sensitivity analysis highlights the high impact of the time / temperature history of the product during the last three links of the cold chain on the final contamination and hence the importance of food retailer practices and consumers behaviour. Results shows also that time / temperature profiles variability has to be taken into account in risk assessment. In further work, the first links of the cold chain, cold room, transport and warehouse and the variation of the load temperature in equipment due to refrigeration cycle (defrost, doors opening) will be integrated in our model. This approach can be used as guideline by industry and public organizations to improve practices, to evaluate the risk and to establish new legislations.

NOMENCLATURE

Aw	product water activity	Q ₀	initial physiological state
b	bacterium index	Q	physiological state
C	thermal capacity (J kg ⁻¹ K ⁻¹)	t	time (s)
CO ₂	concentration of CO ₂ (ppm)	T	product of interest temperature (°C)
E	product characteristics	T _{load}	load temperature (°C)
h ₀	initial physiological state	T _{min}	minimum temperature of growth (°C)
H	heat transfer conductance (W K ⁻¹)	y ₀	initial microbial load of the product (when placed in the display cabinet) (log ₁₀ (CFU) g ⁻¹)
i	product index	y	microbial load (log ₁₀ (CFU) g ⁻¹)
I	itinerary	y _{end}	final microbial load of the product (consumption point) (log ₁₀ (CFU) g ⁻¹)
j	step index	μ _{max}	maximum growth rate (h ⁻¹)
K	number of parameters	μ _{ref}	reference growth rate (h ⁻¹)
l	position index	τ	characteristic time (s)
m	mass of product (kg)		
N	number of simulation runs		
NIT	concentration of nitrite (ppm)		
pH	product acidity		

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