A new tool to control meat products safety: a web based application of predictive microbiology models

Delhalle L., Adolphe Y., Crèvecoeur S., Imazaki P., Daube G. and Clinquart A.

University of Liège, Faculty of Veterinary Medicine, Department Food Science, Sart Tilman, B43bis, 4000 Liège, Belgium

Abstract— Predictive microbiology is considered by the European legislation as a tool to control food safety. Meat and meat products are particularly sensitive to contamination with pathogens. However, development of predictive microbiology models and interpretation of results require specific knowledge. A free web based model has been developed for an easy use by people who are not experts in this field as industries and public authorities. The model can simulate the growth of Salmonella spp, Listeria monocytogenes and Escherichia coli O157 in minced pork meat and on pork meat product (white pudding) under different environmental conditions. The model provides simulations under static or dynamic conditions over time. The user also has the opportunity to import the specific growth rate and cardinal parameters of a bacterium. Unlike polynomial models currently available, this free web access model is distinguished by the use of secondary square roots and primary logistic model with delay. This model permits to have a real time process management, to prospect new formulation for safer products or to design safer processes, to estimate the shelf life of a food product, etc.

 ${\it Keywords} {\color{red} --} {\bf Predictive \quad microbiology, \quad food \quad safety, \\ {\bf modelling.} \\$

I. INTRODUCTION

The overall notification rate in the European Union (EU) in 2008 for *Listeria monocytogenes*, *Salmonella spp.* and verotoxigenic *Escherichia coli* (VTEC)) were respectively 26.4, 0.3 and 0.7 cases per 100,000 population [1]. Meat and meat product are a major source of the notification cases.

A high level of public health protection is one of the fundamental objectives of European food laws. Some food categories possibly posing a high risk to public health are subject to strict criteria. One of the appropriate methods for investigating compliance with the criteria throughout the shelf-life is "predictive"

mathematical modelling established for the food in question" [2].

The microbial population behaviour in foods can be predicted by measuring the effects of environmental factors, assembling the data into a database and synthesising the data into a mathematical model to be incorporated into predictive software [3]. When combined with appropriate monitoring technology, shelf-life or microbial safety can be estimated without recourse to traditional microbiological enumeration techniques.

The objectives of this work were (1) to collect data with standardized microbiological challenge tests (2) to develop and validate a model to simulate the potential growth of *L. monocytogenes*, *Salmonella spp* and *E. coli* O157 in raw minced pork meat and cooked pork meat product (white pudding) under static and dynamic environmental conditions, (3) to create a user friendly interface for people not familiar with predictive microbiology.

II. MATERIAL AND METHODS

A. Experimental design

The experimental design was constructed in order to assess kinetic parameters of pathogens in the minced meat and on white pudding in function of atmospheric conditions and the presence or not of the indigenous flora (Table 1).

B. Challenge tests

Standardized microbiological challenge tests have been used in order to follow pathogens in artificially contaminated meat products at different environmental conditions (NF V01-003, NF V01-009).

A quantity of the minced meat and white pudding batches is irradiated in order to follow pathogens without indigenous flora (Jameson effect).

The products were inoculated with the selected pathogen to obtain 10^2 cells per gram for minced meat or per cm² for white pudding. The minced meat and the white pudding were inoculated in depth and on the surface, respectively.

Table 1 - Experimental design of the challenge tests for the minced meat and white pudding.

Atmospheric condition	Indigenous flora	Bacteria	Temperature (°C)
Under atmospheric air	Presence	L. monocytogenes	4,8,10
	_	Salmonella	8,10,12
	_	E. coli O157	8,10,12
	Absence	L. monocytogenes	4,8,10
	_	Salmonella	8,10,12
	_	E. coli O157	8,10,12
With modified atmosphere	Presence	L. monocytogenes	4,8,10
	_	Salmonella	8,10,12
	_	E. coli O157	8,10,12
	Absence	L. monocytogenes	4,8,10
	_	Salmonella	8,10,12
	_	E. coli O157	8,10,12

The protocols for inoculation had been previously standardized to ensure homogeneous dispersion of the inocula, to check the physic-chemicals properties after inoculation and to limit the variability at a maximum of 5% between batches.

In minced meat, the inoculum has been added at a volume of 1 ml per 100 g of products. The minced meat has been then mixed during 2'30''.

In order to obtain a homogeneous dispersion of the pathogens on the surface, the white puddings have been clamped with mosquito forceps and completely immersed in a solution with the pathogens or sterile water for control during two minutes. The immersed products were transferred over a bin and drained during 15 minutes.

The inoculated minced meat and meat products were packaged in permeable plastic wrap for atmospheric air condition or in high barrier trays and films under modified atmosphere. The trays and film for modified atmosphere packaging were composed of an Ethylene Vinyl Alcohol (EVOH) barrier layer. The

gas composition of packaged trays was $70\% O_2$: $30\% CO_2$ for minced pork meat and $50\% N_2$: $50\% CO_2$ for white pudding.

Samples were stored in a climatic chamber set to the temperature of the challenge test with an accuracy of \pm 1 °C.

The culture and enumeration of micro-organisms (pathogens and total flora) in food samples were performed according to standard methods where available. The enumeration dates have been defined by the nature of the growth test, the nature of the food and conditions of storage.

C. Fitting primary model of data

To estimate the kinetic parameters (maximum growth rate, lag time, initial and maximal concentrations), fitting procedure was performed for each measured flora in each challenge test using the open source software R v2.11.1 (The R Core Team). The Baranyi model with lag phase was used to fit the data.

D. Modelling of pathogens growth

Based on data collected during challenge tests; predictive models simulate pathogens concentration in this food product following environmental conditions (temperature, pH, water activity, modified atmosphere and Jameson effect) . The Figure 1 gives a summary of the methodology used for the development of predictive models.

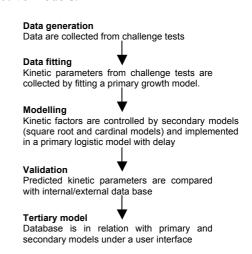


Figure 1 - Steps to construct the tertiary model.

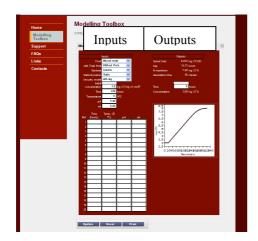
E. Validation

External validation was performed to assess the quality of the predictions of the model by using new data, obtained from challenge tests or growth rate data reported in literature.

The adequacy of a model to predict data can be assessed graphically or on the basis of mathematical and statistical indices (Accuracy and bias factors).

III. RESULTS

Collected data (pathogens and total flora) from 72 challenge tests have been fitted with primary model and kinetic parameters have been implemented in the database. Optimal growth rate has been calculated for each pathogen in function of environmental conditions.



Fiure 2 - Screen capture of the web interface.

The model has been programmed in relation with web interface and the database. The users can select the pathogen, enter their own data, choose between static or dynamic environmental conditions, etc. The Figure 2 is the screen capture of the web interface of the model. The left part of the interface is dedicated for the inputs values and the right part is for the outputs values.

As an example, the Figure 3 gives the graphical evolution the L. monocytogenes concentration in minced meat at three different temperatures with an initial concentration of 1 log CFU/g.

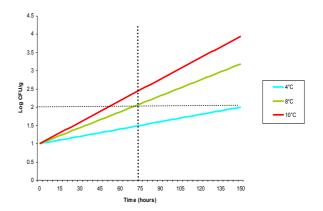


Figure 3. Simulation of *L. monocytogenes* in minced meat with three temperatures

In this example, it could be observed that *L. monocytogenes* grow slowly at 4°C and the food safety criteria (2 log cfu/g) can be respected during the shelf life of the product (72 hours). For the temperature of 8 and 12°C, the concentration of *L. monocytogenes* is over the food safety criteria after 72 hours and minded meat stored at these temperatures present a risk of listeriosis for consumers.

IV. CONCLUSIONS

Predictive microbiology is useful for supporting food safety management systems such as HACCP, Quantitative Risk Assessment and Food Safety Objectives.

This free web based model used cardinals and square root secondary models to simulate the growth of three pathogens in minced pork meat and pork meat product in function of static or dynamic environmental conditions. Standardized challenge tests from one lab are used to give the most reliable database.

In its contribution, the model could offer a powerful tool for industries and public health authorities to control and to manage food safety. It gives knowledge of the microbial ecology of pork product/environment/pathogen combination.

ACKNOWLEDGMENT

This study was conducted with the financial support of the Walloon region (DGO6, Convention n°5713, 2008-2011), Belgium.

REFERENCES

1. European Food Safety Authority. 2010. The Community Summary Report on Trends and Sources of

- Zoonoses. Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2008. European Food Safety Authority, Parma.
- 2. European Parliament and Council of the European Union. 2005. Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Off. J. Eur. Union*: 338/1-338/26.
- 3. McMeekin, T. A. 2007. Predictive microbiology: Quantitative science delivering quantifiable benefits to the meat industry and other food industries. *Meat Science*. 77:17-27.