Predictive models to support manufacturers of processed meat in their compliance with EU regulation 2073/2005

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Introduction

As part of the control measures for *L. monocytogenes,* Food Business Operators (FBO) should conduct studies to identify growth potential of *L. monocytogenes* in products put on the market. Next to the specifications of physicochemical characteristics and available scientific literature, predictive microbiology can be used. Therefore, it is important that existing predictive models are validated for a large category of products and that predictions are compared with results obtained from extensive challenge tests. This study, in cooperation with 30 Belgian companies of processed meat, aimed at the development of a software tool to implement predictive models in the industry to support the compliance with the EU regulation 2073/2005.

Five different categories of meat products were defined: (*i*) cooked meat products with meat structure (e.g. cooked ham), (*ii*) cooked meat products without meat structure, i.e. emulsified meat products (e.g. pate, cooked meat sausage, etc.), (*iii*) raw, salted meat (e.g. bacon), (*iv*) fermented meat products (e.g. salami) and (*v*) aspic meat products (e.g. tongue).

Material and Methods

Based on the data of intrinsic factors provided by and in consultation with the companies, specific recipes for each meat category were established to prepare model products on pilot scale in standardized conditions.

All durability and challenge tests were performed by a BELAC accredited laboratory (LFMFP-UGent) according to the EU technical guidance document on *L. monocytogenes* shelf-life studies for RTE foods (EU CRL, 2008). For each growth curve at constant temperature (7°C) total aerobic count (TAC), lactic acid bacteria (LAB) and *L. monocytogenes* count were analyzed at 15 time points during shelf-life. This was performed for the blanks and two *L. monocytogenes* strains, separately, in monoculture. For growth potential tests, the same parameters were analyzed in threefold on day 0 and at end of shelf-life. Enumeration of *L. monocytogenes* was performed according to ISO 11290-2 using a reduced detection limit. The enumeration of TAC at 22°C was derived from ISO 6222 (4-5 days incubation of PCA at 22°C). LAB was determined according to ISO 15214 (4-5 days incubation of MRS at 22°C). On day 0 and the end of shelf life, the pH, water activity (a_w), dry matter, salt, lactate, acetate and nitrite were determined.

From these extended challenge tests to assess the growth rate, an adaptation factor to the model of Dalgaard and Mejlholm (2009) was determined. As a validation of the adapted kinetic growth model, growth potential tests were performed on five industrial products for each meat category obtained from different companies. Each product was inoculated with a mix of the two *L. monocytogenes* strains and incubated at 4, 7 or 12°C. The intrinsic factors of the products were analysed in threefold at the beginning and end of shelf-life. The microbial analyses were also performed in threefold at the beginning and end of shelf-life and one replicate was analysed at intermediate time points.

The results of this study were compiled in a software program written in Matlab®. The software can be run on a Windows platform after installing the Matlab Runtime that is included in the software.

Results

Cooked meat products (category 1 and 2)
Challenge tests on these products showed that relatively high variability occurred between the different replicates (Fig. 1). This variability was also observed in the challenge tests to determine the growth potential. Between the three replicates of a growth potential challenge test a difference of 2.48 log CFU/g was observed, which indicates the high variability on *L. monocytogenes* even on the same product. According to the EU protocol a growth potential of a challenge test for *L. monocytogenes* should be calculated by the difference between the median on THT and the median on day 0 of the three replicates.

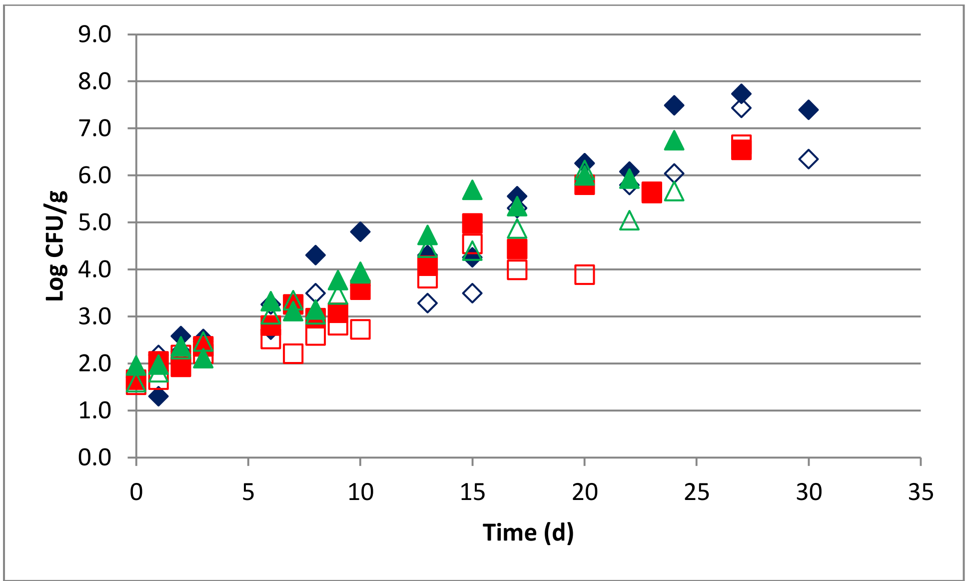

Figure 1: growth of two *L. monocytogenes* strains in three different batches of cooked ham

In this study, several scenarios for growth potential were tested based on the preculturing conditions and time-temperature profiles mentioned in the EU-protocol (Table 1). From the results, it seemed that the growth potential for these different scenario's varied between 2.12 and 5.03. Particularly for the time temperature profile proposed by the EU-protocol, a very high growth potential was obtained.

For this category of products, it was also observed that no interaction (Jameson effect) occurred between the background flora (lactic acid bacteria) and the target organism (Ross et al., 2000). This implies that using a predictive model (e.g. SSSP) including this interaction would lead to significant underestimations of the growth potential (Fig. 2).

Table 1: growth potential of *L. monocytogenes* for different pre-culturing and storage conditions in cooked ham

Inoculum	Pre-culturing	T-profile	Growth potential
L. mono 1	Low T	14d at 4°C + 8d at 8°C	3.73
L. mono 2	Low T	14d at 4°C + 8d at 8°C	3.68
Cocktail	Low T	7d at 8°C + 15d at 12°C	5.03
Cocktail	Optimal T	14d at 4°C + 8d at 8°C	2.12
Cocktail	Low T	14d at 4°C + 8d at 8°C	2.98
Cocktail	Low T	24d at 4°C + 12d at 8°C	2.46

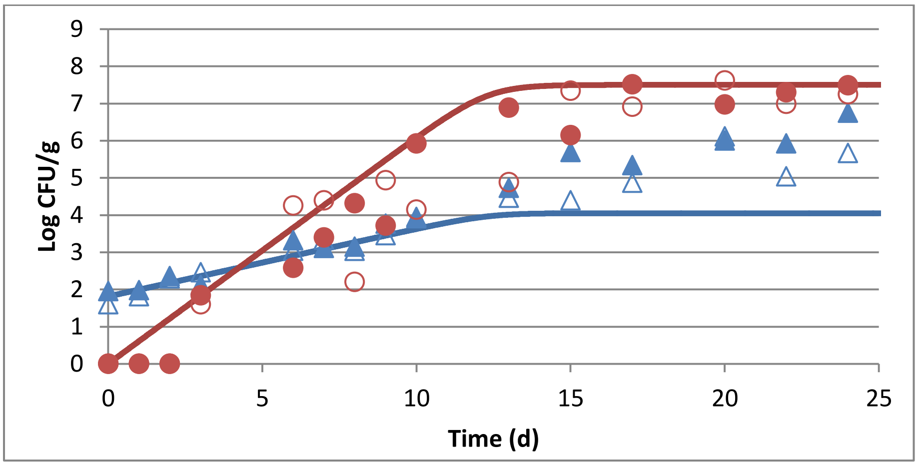

Figure 2: growth of two *L. monocytogenes* strains (triangles) and lactic acid bacteria (circles) on cooked ham together with the growth predictions by SSSP for lactic acid bacteria (red line) and *L. monocytogenes* (blue line)

Raw salted meat (category 3) and fermented meat (category 4)

At least 5 challenge tests were performed on industrial products for both categories. In none of these cases, growth of *L. monocytogenes* occurred, indicating that these products are not at high risk for growth of this pathogen. By consequence, it was also not possible to develop or validate a model for these products.

Aspic products (category 5)

The aspic products, which contain different meat pieces in a jelly structure, are characterized by a large range of pH-values. This implies that in some of the products growth of *L. monocytogenes* was possible while in other products not. The inter- and intrabatch variability on the *L. monocytogenes* growth was also significant. However, the growth potential was much lower than for the cooked meat products (Table 2). This is partly due to the intrinsic and extrinsic factors of the meat and partly due to the interaction with the background flora.
In general, the prediction in this category was better when the interaction with the lactic acid bacteria was incorporated into the model. Therefore, the option of background flora was included in the software for this module.

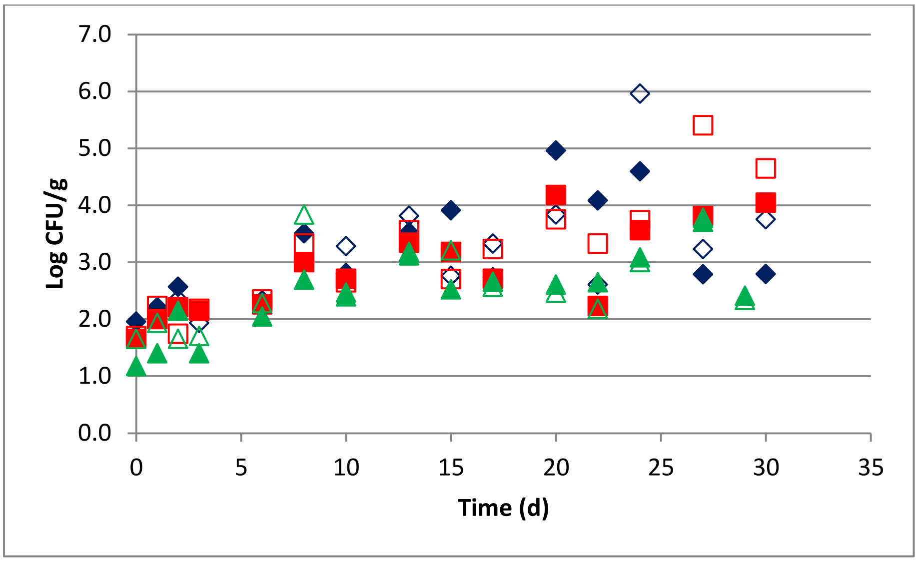

Figure 3: growth of two *L. monocytogenes* strains in three different batches of an aspic product

Table 2: growth potential of *L. monocytogenes* for different pre-culturing and storage conditions in aspic products

Inoculum	Pre-culturing	T-profile	Growth potential
L. mono 1	Low T	14d at 4°C + 8d at 8°C	2.38
L. mono 2	Low T	14d at 4°C + 8d at 8°C	1.06
Cocktail	Low T	7d at 8°C + 15d at 12°C	1.72
Cocktail	Optimal T	14d at 4°C + 8d at 8°C	1.99
Cocktail	Low T	14d at 4°C + 8d at 8°C	1.15
Cocktail	Low T	28d at 4°C + 14d at 8°C	1.18

Description of the software

Before starting the actual simulations, the software-user has to select a specific meat category for which he wants to study the effect of certain inputs. As described above, five different categories have been selected. Within the Listeria Meat Model, Category 1 and Category 2 are combined in one software model, focusing on Listeria growth in cooked meat products.

The software simulates the growth dynamic of *L. monocytogenes* taking into account: (*i*) the product-specific properties, (*ii*) the environmental conditions and (*iii*) the interaction with lactic acid bacteria, in case of aspic products.
The first important input is the initial concentration of *L. monocytogenes*. The target value immediately after production is absence in 25 g (-1.4 log CFU/g). This initial concentration influences only the time at which the limit of 100 CFU/g will be exceeded (Fig. 4). The inputs of the product characteristics are based on the most important factors influencing the growth of *L. monocytogenes* in meat products: salt, pH, residual nitrite, acetic acid/acetate, diacetate, lactic acid/lactate. These data need to be specified on product basis by the user and are automatically converted to water phase basis by the

software, based on the dry matter (Fig. 4). A specific temperature profile with an unlimited amount of steps can be specified. The model is, however, only validated for the cold chain (temperatures < 15°C). The software allows higher temperatures but warns the user that this is beyond the validated range for temperature.
Another important factor for products packed under modified atmosphere is the amount of dissolved CO_2 at equilibrium. The amount of dissolved CO_2 is dependent on the initial CO_2 concentration in the headspace, the storage temperature and the gas/product ratio (Devlieghere et al., 1998). For those cases where the user does not know the amount of dissolved CO_2, a CO_2 calculator is included in the software.
For some inputs (inoculum, salt concentration, pH, dry matter and temperature) some uncertainty values can be used. It should be noted that combining a lot of uncertainties on the input values could lead to a very large confidence interval around the predicted growth.
The software offers the possibility to compare two different products or two different temperature profiles. These can be represented together on the output (Fig. 4).

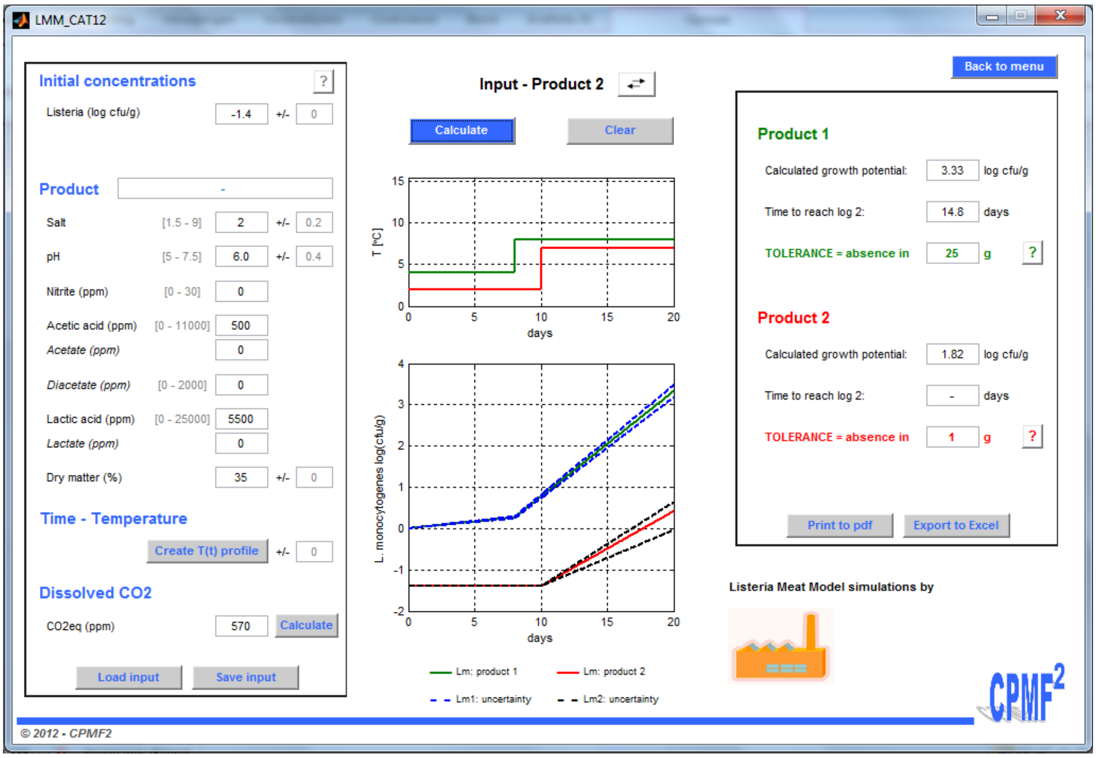

Figure 4: Print screen of ListeriaMeatModel for cooked meat products for two different products and T-profiles.

The software outputs, on the one hand, a graphical representation on the growth of *L. monocytogenes* as a function of time and, on the other hand, a numerical output. This latter gives (*i*) the growth potential, the difference between the cell count at the end of shelf life and the initial *Listeria* concentration, (*ii*) the time to reach 2 log CFU/g (legal criterion) and (*iii*) the tolerance on the day of production. This tolerance is determined by the calculated growth potential and gives to which extent the target at day 0 (absence in 25 g) can be extended (Table 3) (Vermeulen et al., 2011). If this criterion is reached, it can be avoided that the limit of 2 log CFU/g is exceeded at the end of shelf-life.

Table 3: tolerance criteria on day 0 for different growth potentials

Growth potential according EU protocol (log CFU/g)	Tolerance on day 0 (microbial criterium)
Between -1.00 en 0.00	Absence in 0.01 g
Between 0.00 en 0.49	Absence in 0.01 g
Between 0.50 en 0.99	Absence in 0.1 g
Between 1.00 en 1.99	Absence in 1 g
Between 2.00 en 2.99	Absence in 10 g
> 2.99	Absence in 25 g

Conclusions

For the model building, the results for Category 1 & Category 2 (cooked meat products) were considered together as growth dynamics of *L. monocytogenes* were identical in both classes. For Category 3 and 4, all performed challenge tests showed that no growth of *L. monocytogenes* occurred. Therefore, no model could be used and only information sheets are added to the software. A separate model was built for Category 5 as the growth properties clearly differed from these in the cooked meat products and the interaction effect with lactic acid bacteria was built in.

The developed model is based on a thoroughly validated predictive model and can be used as an alternative method for challenge testing. For companies the use of this software can be cost-effective as the amount of microbial analyses can be reduced. Besides the software can be time-saving in product innovation, as durability and challenge test are time consuming and labor intensive. For more information regarding the software CPMF² can be contacted (info@cpmf2.be)

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