PE6.03 A predictive model for growth of L. monocytogenes in meat products with seven different hurdle variables 104.00

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Abstract: **The objective of this study was to create a model that predicts the growth of *Listeria monocytogenes* in ready-to-eat meat products with addition of up to seven different chemical or physical hurdles. A total of 446 growth curves generated from growth of *Listeria monocytogenes* on surfaces of meat products with different concentrations/conditions of the variables were used to train an artificial neural network (ANN). The ANN generated a model that predicts the maximum specific growth rate for combinations of the seven variables. The performance of the model was measured by comparing predicted and observed values of the specific growth rates from a validation data set. A total of 64 new growth curves were obtained for validation. For the growth curves with specific growth rate higher than 0.001 h^{-1}, the model has a bias factor of 0.79 and an accuracy factor of 1.39.**

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Index Terms—predictive modeling, *L. monocytogenes*, meat products, artificial neural network

I. INTRODUCTION

THE Danish meat industry requires predictive tools that can facilitate the work with microbial safety assessment in ready-to-eat (RTE) meat products.

The use of predictive models requires good performance of the models, relevant environmental and physical variables, easy access to the models, and a user-friendly interface.

At the Danish Meat Research Institute (DMRI), it was decided to develop at model capable of predicting the growth of *L. monocytogenes* in RTE meat products because, in accordance with EU legislation [2], the number of *L. monocytogenes* in stabilized RTE meat products must not exceed 100 CFU/g. In accordance with US legislation [4], products for export to the United States must be stabilized with sodium lactate or sodium diacetate or must be heat-treated after handling. This legislation implies that the meat industry requires tools to reduce the occurrence of *L. monocytogenes* because it is widespread in the environment and because the RTE meat products can be recontaminated during handling, slicing and packaging [8,11].

When producing RTE meat products, the Danish meat industry operates with up to seven hurdles in order to prevent growth of *L. monocytogenes*: pH, NaCl, sodium lactate, sodium acetate, sodium nitrite storage temperature and CO_2 in the packaging atmosphere The meat industry requires a model that can predict the growth of *L. monocytogenes* in RTE meat products to which different combinations of these seven hurdles are added in order to obtain recipes that prevent or minimize growth of *L. monocytogenes.*

Existing predictive models [1,3], (Pathogen Modelling Program (PMP) and Combase Predictor (CP)) include 4-5 variables ,and are developed from data obtained in broth. It has been shown that at 25°C, the growth limits for pH and a_w are lower in broth than on solid surfaces [7]. These results indicate that it is very important to use data generated from experiments performed on solid surfaces when developing predictive models for growth of *L. monocytogenes* in RTE meat products. Therefore, this predictive model is based on growth data from experiments performed on solid surfaces of agar and RTE meat products with different levels of pH, NaCl, sodium lactate, sodium acetate, sodium nitrite storage temperature and CO_2 in the packaging atmosphere.

The objective was to develop a model capable of predicting the maximum specific growth rate (μ_{max}) as a function of all hurdle conditions in a worst-case scenario.

II. MATERIALS AND METHODS

A. Model

The data for developing and training the ANN model were generated in meat products. A total of 446 growth curves generated from growth of *L. monocytogenes* on surfaces of different meat products were selected for the model. For each growth curve, the log cfu/g was plotted against time, the measuring points were fitted with the

DMFIT program [5], and μ_{max} was calculated.

The 446 calculated μ_{max} values and the matching levels of the hurdles were used to train the ANN using the network architecture with seven input neurons, three hidden neurons, and a single output neuron. Training was performed with a standard back propagation of error algorithm using software developed at DMRI.

B. Validation

In order to validate the predictive model, 64 new growth curves for *L. monocytogenes* on solid surface were generated. Pasteurised meat products (saveloys) with 24 different combinations of hurdles in a balanced setup were produced at DMRI, spiked with *L. monocytogenes,* and stored at four different temperatures. The observed and calculated μ_{max} values were compared.

The performance of the model was measured by comparing predicted (P) and observed (O) values. [9] gave the following equations for the bias factor, B_f, and accuracy factor, A_{f1}:

$$B_f = 10^{\frac{1}{n}\sum_{i=1}^{n}\log\left(\frac{\mu_{predicted}}{\mu_{observed}}\right)}$$

$$A_{f1} = 10^{\frac{1}{n}\sum_{i=1}^{n}\left|\log\left(\frac{\mu_{predicted}}{\mu_{observed}}\right)\right|}$$

[6] modified the accuracy factor to:

$$A_{f2} = 10^{\sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(\log\left(\frac{\mu_{predicted}}{\mu_{observed}}\right)\right)^2}}$$

In order to compare the accuracy factors obtained in this study with those obtained in other studies, equations both from [9] and [6] were used.

III. RESULTS AND DISCUSSION

The observed and predicted values for μ_{max} are shown in Figure 1, and bias and accuracy are tabulated in Table 1. As can be seen from Figure 1, circle 1, the DMRI model underestimates μ_{max} in 3 cases where the growth is relatively fast. Similarly, the model underestimates μ_{max} in 4 cases with specific growth rates of around 0.0065, which correspond to doubling times of approx. 106 h (circle 3). In contrast, the DMRI model overestimates in 3 cases (circle 2), where the growth is very slow and μ_{max} is almost equal to zero.

Figure 1. Predicted specific growth rate (μ_{max}) versus observed specific growth rates (μ_{max}) for all cases in the validation.

One of the major problems that arises when attempting to create a model encompassing both growth and no-growth hurdle combinations is that there are innumerable hurdle combinations that result in outcomes with very slow growth or no-growth, whereas there are few optimal hurdle conditions resulting in fast growth. This means that any training set will always be poorly balanced. Fortunately, hurdle combinations giving rise to optimal or near-optimal growth conditions for *L. monocytogenes* will never be considered for use in RTE meat products produced on a large-scale. Therefore, it is of little consequence that the model slightly underestimates growth rates in experiments with very fast growth corresponding to the experiments shown in circle 1, Figure 1.

The most problematic outliers are in circle 3, Figure 1, in particular one point with observed μ_{max} = 0.006 and predicted μ_{max} = 0.0018. The experiment responsible for this outlier had growth conditions on the boundary between growth and no-growth. The outlier might be explained by lack of data with similar hurdle combinations in the training data.

In Table 1, the bias, bias factor, accuracy and accuracy factor are calculated for the cases where the observed growth rates are higher than 0.001 h^{-1}. When validating this model, the limit between growth and no-growth was arbitrarily set to μ_{max} (observed) = 0.001 h^{-1}. From the definitions of bias and accuracy in Table 1, it is obvious that these parameters cannot be estimated if no-growth has occurred (observed μ_{max} = 0). In order to compare the obtained bias and accuracy with data from other studies, only cases where growth occurred can be used.

Table 1. Bias and accuracy for the μ_{max}

The perfect predictive model has A_f = B_f = 1. The DMRI model has B_f = 0.79 (see Table 1). The consequences can be evaluated from Figure 1 - the model overpredicts the growth rate when no-growth is observed and tends to underestimate the growth rate when growth is observed.

The accuracy factor of the model based on the validation data where growth is observed is A_{f1} = 1.39 (Table 1). [10] state that A_f can be expected to increase by 0.10-0.15 for each variable. As the DMRI model has seven hurdle variables, A_f would be expected to be between 1.70 and 2.05. The observed accuracy factor is therefore acceptable.

The PMP and Food Micro Model (FMM) have been validated in relation to meat products (n=92) with the variables temperature, pH and a_w [12]. For the PMP and FMM, the bias factors were 1.33 and

1.35, respectively. The values can be compared with B_f = 0.79 for the DMRI model. The accuracy factors calculated from the original formula by [9] were 1.74 and 1.73, respectively. These values can be compared with A_{fl} = 1.39 for the DMRI model.

Comparison of predictions (Table 2) from the DMRI model, the PMP and the CP results in substantial differences in the growth rates. The differences in the growth rates could be explained by the fact that the growth rates are higher in broth than on RTE meat product surfaces [7].

Tabel 2. Predicted specific growth rates (log 10_{conc})/h for *L. monocytogenes* from PMP, Combase Predictor and DMRI model.

Furthermore, the DMRI model is corrected for the natural content of lactate, as the natural content of lactate in meat is added to the input value of lactate. In the DMRI model, the values of sodium chloride, acetate and lactate concentrations are converted to the concentrations in the water phase, because the substances are poorly solubilized in the fat fraction of the product. These corrections ensure that the hurdle concentrations are closer to the correct concentrations in the RTE meat products, and might explain some of the differences between the predictions from the three models.

Comparison of predictions (Table 2) from the DMRI model, the PMP and the CP at high hurdle levels (4°C or 6°C, 3.4% NaCl and 90 ppm nitrite) are tabulated in Table 2. The μ_{max} predicted with the DMRI model are two to eight times lower than the μ_{max} predicted with the CP, where the largest difference is seen at the highest hurdle level (pH 5.8). Compared with the PMP model, the growth rates are four to 14 times lower for the DMRI model.

At a lower hurdle level (7°C, 1 % or 2% NaCl and 0 or 20 ppm nitrite), the differences in μ_{max} are reduced. The μ_{max} values predicted with the DMRI model are a factor of two lower than PMP, and almost equal to CP's predictions.

The predicted values tabulated in Table 2 can also be compared with experiments performed on meat surfaces. A search in the Combase database resulted in 12 experiments with ham spiked with *L. monocytogenes*. The data were fitted with DMFIT [5]. The conditions were: pH 6.2, NaCl 3.4% and nitrite 90 ppm and a storage temperature of 4°C or 6°C, which are very close to the conditions for the predictions in Table 3. At 4°C, four experiments resulted in no-growth and two experiments had a μ_{max} of 0.0024 and 0.0013. At 6°C, six experiments showed no-growth. These results indicate that the DMRI model results in more realistic predictions at high hurdle levels.

The model is available online. The user-interface (Figure 2) is divided in two sections: 1. The input section, in which the user adds the values for a specific product. 2. The result section, in which the model returns a growth curve, specific growth rate and doubling time for *L. monocytogenes* in relation to the selected values in section 1.

Figure 2. The user-interface of the DMRI model

IV. CONCLUSION

A predictive model for the growth of *L. monocytogenes* in pasteurized RTE meat products was developed based on an ANN. In this model, growth of *L. monocytogenes* in pasteurised meat products with addition of up to seven different hurdles can be predicted. Upon validation of the model using data obtained from growth of *L. monocytogenes* on solid surfaces of saveloys, the model showed satisfactory performance, with a bias factor of 0.79 and accuracy factor of 1.39. Compared with other publicly available predictive models (Pathogen Modelling Programme and Food Micro Model), the DMRI model performs just as well, though the model could be improved by increasing the number of experiments in which growth of *L. monocytogenes* is very slow. The predictive model is available online.

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Table 1. Bias and Accuracy for the maximum specific growth rate in cases where growth was observed.

Bias	$B = \frac{1}{n}\sum_{i=1}^{n}\log\left(\frac{\mu_{predicted}}{\mu_{observed}}\right)$	-0.10
Bias factor	$B_f = 10^{B}$	0.79
Accuracy	$A_1 = \frac{1}{n}\sum_{i=1}^{n}\left\|\log\left(\frac{\mu_{predicted}}{\mu_{observed}}\right)\right\|$	0.14
	$A_2 = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(\log\left(\frac{\mu_{predicted}}{\mu_{observed}}\right)\right)^2}$	0.19
Accuracy factor	$A_{f1} = 10^{A_1}$	1.39
	$A_{f2} = 10^{A_2}$	1.54

Table 2. Predicted specific growth rate (log 10_{conc})/h for *L. monocytogenes* from PMP, CP and DMRI model.

	PMP (h^{-1})	CP (h^{-1})	DMRI (h^{-1})
4°C, 3.4% NaCl, 90 ppm nitrite:			
pH 5.8	0.0127	0.0072	0.0009
pH 6.2	0.0163	0.0097	0.0027
6°C, 3.4% NaCl, 90 ppm nitrite:			
pH 5.8	0.0174	0.0109	0.0026
pH 6.2	0.0227	0.0147	0.0060
7°C, 2% NaCl, pH 6.2:			
20 ppm nitrite	0.0391	0.0257	0.0177
0 ppm nitrite	0.0431	0.0274	0.0213
7°C, 1% NaCl, pH 6.2:			
20 ppm nitrite	0.0443	0.0268	0.0246
0 ppm nitrite	0.0478	0.0285	0.0292

Figure 1. Predicted specific growth rate (μ_{max}) versus observed specific growth rate (μ_{max}) for all cases in the validation.

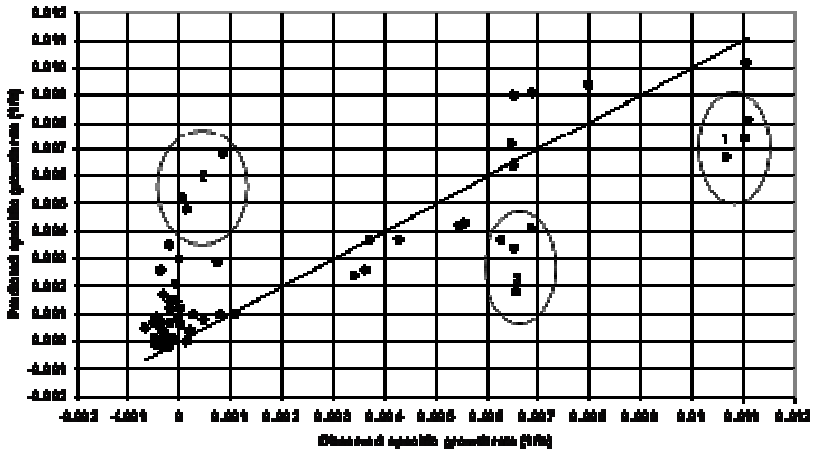

Figure 2. The user-interface of the DMRI model