Chapter 20

Modeling Growth of *Listeria* and Lactic Acid Bacteria in Food Environments

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Abstract

Predictive food microbiology models can facilitate the assessment and management of microbial food safety. Importantly, the combined effect of storage conditions and product characteristics can be predicted by successfully validated models. This makes it easier and faster to develop or reformulation safe food recipes and predictions can be used to documents safety of available foods. The effect of various product characteristics and storage conditions must be taken into account and extensive mathematical models including the effect of these environmental factors are needed. Here the development, evaluation and application of an extensive growth and growth boundary model for *Listeria monocytogenes* including the effect of 12 environmental factors as well as the growth dampening effect of lactic acid bacteria is described. The Food Spoilage and Safety Predictor software is used to illustrate how predictions can be applied.

Key words Predictive food microbiology, Simplified cardinal parameter models, Interaction between environmental factors, ψ-Value, Microbial interaction, Application software

1 Introduction

Bacterial pathogens are of major importance for safety of numerous fresh and lightly preserved foods and their growth is essential to assess and manage. This is particularly important when products are formulated, reformulated, and when packaging or conditions in the food chain are changed. Prediction of growth depending on food processing, storage conditions (temperature, atmosphere) and product characteristics (salt/a_w, pH, organic acids, smoke components, competing microbiota) can facilitate the assessment and management of food safety. This is interesting as storage conditions and product characteristics (model input) and predictions can be obtained more rapidly and less costly than the series of challenge tests and storage trials required to determine safe shelf-life and safe product recipes (Fig. 1).

To support the assessment and management of food safety, predictive food microbiology growth models must provide

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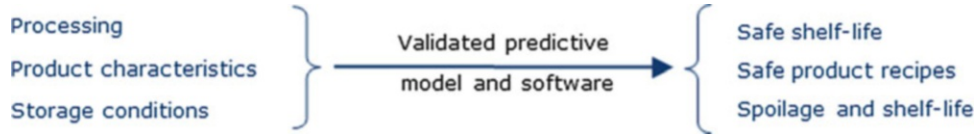

Fig. 1 Overview of application for validated predictive food microbiology models and software in product development and in documentation of food safety and quality

predictions similar to those observed in naturally contaminated food [1, 2]. For this to be possible, growth and growth boundary models must include the effect of all the conditions in food that has an important effect on growth of the relevant bacterial pathogens [3, 4]. This include several storage and product characteristics, interaction between these environmental factors and for some products the effect of microbial interaction between the bacterial pathogen and the food microbiota [5–7]. Prior to practical use in assessment and management of food safety, predictive food microbiology models must be evaluated to determine their range of application where they have been successfully validated [5].

Here we demonstrate how separate growth and growth boundary models for *Listeria monocytogenes* and psychrotolerant lactic acid bacteria (LAB) were developed to include the effect of 12 environmental factors, interaction between these factors and the growth dampening effect of high concentrations of LAB on growth of the pathogen. The successfully validated models have been included in the Food Spoilage and Safety Predictor (FSSP) software to facilitate their practical use.

2 Methods and Models

2.1 Predictive Food Microbiology

Primary growth models describe changes in cell concentration during the storage time whereas secondary growth models describe the effect of environmental factors, including storage conditions and product characteristics, on key parameters in primary models for example the growth rate. Large amounts of data are needed to develop secondary growth models for several storage and product characteristics and automated growth analyzers, based on absorbance measurements, are often used to quantify the effect of environmental factors on growth rates [8]. Various user-friendly predictive food microbiology application software are available [9]. They include fitted secondary models and predictions are obtained by using storage conditions and product characteristics as model input. The fitted secondary models then predict values for growth kinetic parameters that are used in primary models to predict growth during storage by the application software (*see* **Note 1**).

Table 1
Logistic model with delay[a]

Time	Differential form	Integrated and log(10)-transformed form
$t < \lambda$	$\frac{dN}{dt} = 0$	$\log(N_t) = \log(N_0)$
$t \geq \lambda$	$\frac{dN}{dt} = N \cdot \mu_{max}\left[1 - \frac{N_t}{N_{max}}\right]$	$\log(N_t) = \log\left(\frac{N_{max}}{1+\left[\frac{N_{max}}{N_0}-1\right]\cdot\exp(-\mu_{max}\cdot(t-\lambda))}\right)$

[a]"t" is the time of storage and "N_0" the initial cell concentration (cfu/g). Other parameters are explained in the text. The Logistic model is a classical growth model. The parametrization shown here with delay is widely used within predictive food microbiology [28]

2.2 Primary Models for Growth and Microbial Interactions

Primary growth models for changes in cell concentration (N, cfu/g) during storage include kinetic parameters for lag time (λ, h), maximum specific growth rate (μ_{max} $1/h$) and maximum population density (N_{max}, cfu/g) (Table 1). Classical competition models including the Lotka–Volterra model can be used to describe simultaneous growth of bacterial pathogens and other microorganisms in food but simpler primary models based on the differential form of the logistic equation are used increasingly within predictive food microbiology (Eq. 1, [7, 10–12]). These simpler interaction models can describe the Jameson effect where growth of *L. monocytogenes* stops when concentrations of LAB reach their maximum population density (Fig. 5). It can be important to predict the effect of microbial interaction, particularly, for products where high concentration of LAB does not result is sensory spoilage of products. These predictions require accurate prediction of growth for both LAB and *L. monocytogenes* as well as an interaction model to quantitatively describe the growth dampening of *L. monocytogenes* by LAB (Eq. 1).

$$\begin{cases} t < \lambda_{Lm}, & \dfrac{dLm/dt}{Lm_t} = 0 \\ t \geq \lambda_{Lm}, & \dfrac{dLm/dt}{Lm_t} = \mu_{maxLm} \cdot \left(1 - \dfrac{Lm_t}{Lm_{max}}\right) \cdot \left(1 - \dfrac{CF \cdot LAB_t}{LAB_{max}}\right) \\ t < \lambda_{LAB}, & \dfrac{dLAB/dt}{LAB_t} = 0 \\ t \geq \lambda_{LAB}, & \dfrac{dLAB/dt}{LAB_t} = \mu_{maxLAB} \cdot \left(1 - \dfrac{LAB_t}{LAB_{max}}\right) \end{cases} \quad (1)$$

where Lm and LAB, both >0 cfu/g, signify concentrations of *L. monocytogenes* and LAB, respectively, and CF is a competition factor that allows the predicted cell concentration of *L. monocytogenes* to increase (CF < 1) or decrease (CF > 1) after the cell concentration of LAB has reached their maximum

Table 2
Parameter values in secondary growth rate (μ_{max}) models (Eq. 2)

	Listeria monocytogenes			Lactic acid bacteria		
Model parameter	**Value**	n_1	n_2	**Value**	n_1	n_2
μ_{ref} $(1/h)$	0.419	–	–	0.583	–	–
T_{min} (°C)	−2.83	–	–	−5.25	–	–
$a_{w\ min}$	0.923	–	–	0.928	–	–
pH_{min}	4.97	–	–	4.24	–	–
Phenol (P_{max}, ppm) (ppm)	32	–	–	40.3	–	–
$CO_{2\ max}$ (ppm)	3140	–	–	6691	–	–
Nitrite (MIC_{NIT}, ppm)	350	–	–	2780	–	–
Minimum inhibitory concentrations (MIC) of undissociated organic acids (mM)						
Acetic acid	10.3	0.5	1	151.3	0.5	1
Benzoic acid	0.35	1	1	1.51	1	2
Citric acid	2.12	1	1	10.3	1	2
Diacetate	4.80	0.5	1	33.3	0.5	1
Lactic acid	3.79	1	1	12	1	1
Sorbic acid	1.9	1	1	12.6	1	2

population density (LAB_{max}) [12]. Growth rates of *L. monocytogenes* ($\mu_{max\ Lm}$) and LAB ($\mu_{max\ LAB}$) can be obtained from secondary growth rate models (Eq. 2, Table 2) and with a constant CF value of 1.0, corresponding to the Jameson effect, Eq. 1 include no parameter values that need to be fitted from studies of microbial interaction [10].

2.3 Simplified Cardinal Parameter Models for Growth Rate

Cardinal parameter models (CPM) rely on the gamma-concept [13, 14] where μ_{max} at optimal growth conditions ($\mu_{max\text{-}opt}$) is reduced by each of the environmental factors that differ from the optimal growth condition. The effect of each sub-optimal environmental factor is expressed by a term with a value between 0 and 1 and the combined effect of several environmental terms is determined by multiplication of their respective values between 0 and 1. Le Marc et al. [15] expanded the gamma-concept by introducing a term (ξ) to take into account the effect of interaction between environmental factors (Eqs. 3–5). The simplified CPM presented here uses the Le Marc-approach and differs from classical CPM by taking into account exclusively temperature and pH values below

the growth optimum for these environmental factors (Eq. 2). Furthermore, $\mu_{\text{max-opt}}$ in classical CPM is replaced with μ_{ref} determined at a lower reference temperature of 25 °C. Eq. 2 includes 12 environmental factors: Temperature, salt/water activity, pH, smoke components/phenol, carbon dioxide, nitrite, diacetate, and acetic, benzoic, citric, lactic, and sorbic acids. An extensive secondary μ_{max} –model has been developed for both *L. monocytogenes* and psychrotolerant LAB [4, 16]. In both cases the models were gradually expanded by combining environmental-factor-terms form different studies [6, 10, 11, 16].

$$\mu_{\max}=\mu_{\text{ref}}\cdot\left(\frac{T-T_{\min}}{T_{\text{ref}}-T_{\min}}\right)^2\cdot\left(\frac{a_w-a_{w\min}}{1-a_{w\min}}\right)\cdot\left(1-10^{(pH_{min}-pH)}\right)\cdot\left(\frac{P_{\max}-P}{P_{\max}}\right)\cdot$$
$$\left(\frac{CO_{2\max}-CO_{2\text{equilibrium}}}{CO_{2\max}}\right)\cdot\left(\frac{MIC_{NIT}-NIT}{MIC_{NIT}}\right)\cdot\left(1-\left(\frac{AAC_U}{MIC_{U\,AAC}}\right)^{n1}\right)^{n2}\cdot$$
$$\left(1-\left(\frac{BAC_U}{MIC_{U\,BAC}}\right)^{n1}\right)^{n2}\cdot\left(1-\left(\frac{CAC_U}{MIC_{U\,CAC}}\right)^{n1}\right)^{n2}\cdot\left(1-\left(\frac{DAC_U}{MIC_{U\,DAC}}\right)^{n1}\right)^{n2}\cdot\left(1-\left(\frac{LAC_U}{MIC_{U\,LAC}}\right)^{n1}\right)^{n2}$$
$$\left(1-\left(\frac{SAC_U}{MIC_{U\,SAC}}\right)^{n1}\right)^{n2}\cdot\xi \tag{2}$$

where μ_{ref} is equal to $\mu_{\max}$ at the reference temperature (T_{ref}) of 25 °C; T is the storage temperature (°C); $T_{\min}$ is the theoretical minimum temperature (°C) that prevents growth; a_w is water activity calculated from the concentration of NaCl in the water phase of the product (WPS), using the relationship a_w = 1–0.0052471*-WPS-0.00012206*WPS^2 [17]; P is the concentration (ppm) of smoke components (phenol); $CO_{2\ \text{equilibrium}}$ is the concentration (ppm) of dissolved CO_2 at equilibrium; NIT is the concentration (ppm) of nitrite and MIC_{NIT} is the minimum concentration (ppm) of nitrite that prevents growth; AAC_U, BAC_U, CAC_U, DAC_U, LAC_U, and SAC_U are the concentrations (mM) of undissociated acetic acid, benzoic acid, citric acid, diacetate, lactic acid and sorbic acid, respectively, in the water phase of the product; $MIC_{U\ AAC}$, $MIC_{U\ BAC}$, $MIC_{U\ CAC}$, $MIC_{U\ DAC}$, $MIC_{U\ LAC}$ and $MIC_{U\ SAC}$ are the fitted minimum concentrations (mM) of undissociated acetic, benzoic, citric, diacetate, lactic and sorbic acids, respectively, that prevent growth (*see* Subheading 2.4).

To take into account interaction between all the environmental factors, the applied Le Marc approach uses each environmental term to calculate a φ value as shown in Eq. 3 with the temperature term from Eq. 2 as an example. The combined effect of all environmental terms is then expressed as a ψ-value calculated from Eq. 4 [15]. ψ-values divide the space of growth conditions into three

regions (Eq. 5). ψ-values <0.5 corresponds to growth and no interaction between environmental factors. With $0.5 < \psi \leq 1.0$ growth occurs but μ_{max} values are reduced due to interaction between environmental factors. The growth boundary has $\psi = 1.0$ and for $\psi \geq 1.0$ no growth occurs (Fig. 6).

$$\varphi_{\text{Environmental term}} = \left(1 - \sqrt{\text{Environmental term}}\right)^2$$
$$\varphi_T = \left(1 - \frac{(T - T_{\min})}{(T_{\text{ref}} - T_{\min})}\right)^2 \quad (3)$$

$$\psi = \sum_i \frac{\varphi_{c_i}}{2 \prod_{j \neq i} \left(1 - \varphi_{c_j}\right)} \quad (4)$$

$$\xi(\varphi(T, a_w, \text{pH}, P, CO_2, \text{NIT}, \text{AAC}, \text{BAC}, \text{CAC}, \text{DAC}, \text{LAC}, \text{SAC})) = \begin{cases} 1 & , \Psi \leq 0.5 \\ 2(1 - \Psi) & , 0.5 < \Psi < 1 \\ 0 & , \Psi \geq 1 \end{cases} \quad (5)$$

2.4 Cardinal Parameter Values and Secondary Model Terms for Environmental Factors

1. Mixtures of four isolates of both *L. monocytogenes* (Lm-mix) and of psychrotolerant LAB/*Lactobacillus sakei* (LAB-mix), all previously isolated from seafood, were used for determination of cardinal parameter values including minimum inhibitory concentrations (MIC) for nitrite and organic acids. A limited number of isolates were used for model development and a large number of isolates were used in product validation studies including challenge tests and studies of naturally contaminated products [4, 6, 7, 11].
2. Here, the growth inhibiting effect of six to 11 different concentrations of nitrite and undissociated diacetate and acetic, benzoic, citric, lactic, and sorbic acids was determined at 8 °C and pH 6.0 by using brain heart infusion (BHI) broth for *L. monocytogenes* and APT broth for LAB. Salts of organic acids was used to improve solubility and concentrations (mM) of undissociated organic acids were calculated from Eq. 6, using pK_a values of 4.76, 4.19, 3.13, 3.86, and 4.76 for acetic/diacetate, benzoic, citric, lactic, and sorbic acids, respectively.

$$\text{Concentration of undissociated organic acid (mM)} = \frac{\text{Total conc. of organic acid}}{1 + 10^{\text{pH} - pK_a}} \quad (6)$$

3. For each concentration of nitrite and of the different organic acids, inoculation levels of 10^1, 10^2, 10^3, 10^4, and 10^5 cfu/ml

were examined in triplicate by automated absorbance measurements at 540 nm using an automated microplate reader. The detection time, defined as a relative change in absorbance of 0.05 at 540 nm, was determined from each of the obtained absorbance curves. μ_{max} values for Lm-mix and LAB-mix at each concentration of the different organic acids were determined from detection times by using Eq. 7.

$$\text{Ln (inoculation level, cfu/}g) = \text{Constant} - \mu_{max}(1/h) \cdot \text{Detection time}\,(h) \tag{7}$$

4. MIC values of undissociated organic acids ($MIC_{U\ OA}$) were determined by fitting Eq. 8 to square root-transformed μ_{max} values and studied concentrations of undissociated organic acids (OA_U, mM). When fitting Eq. 8), $n1$ was set to fixed values of 0.5 or 1.0 and $n2$ was set to 1.0 or 2.0 in order to describe data most appropriately and this was determined from root mean square error (RMSE) values. Selected $MIC_{U\ OA}$, $n1$, and $n2$ values (Table 2) were used in Eq. 2 (*see* **Note 2**).

$$\sqrt{\mu_{max}} = \sqrt{\mu_{ref_{8^\circ C}} \cdot \left(1 - \left(\frac{[OA_U]}{MIC_{U\ OA}}\right)^{n1}\right)^{n2}} \tag{8}$$

5. For *L. monocytogenes* the cardinal parameter values $a_{w\ min}$ (0.928), pH_{min} (4.97), $CO_{2\ max}$ (3140), and MIC_{NIT} (350) as shown in Table 2 were taken from previous studies [10, 18]. In the same way for psychrotolerant LAB cardinal parameter values for pH_{min} of 4.24 and for $a_{w\ min}$ of 0.928 originated from Wijtzes et al. [19] and $CO_{2\ max}$ of 6691 ppm were determined by Devlieghere et al. [20].

2.5 Product Calibration of Secondary Growth Rate Models

With cardinal parameter values from studies in liquid laboratory substrates and from available publications (*see* Subheading 2.4) the next step includes product calibration of the growth rate model (Eq. 2). In this step the value of a few key parameters including μ_{ref}, T_{min}, and P_{max} are determined from μ_{max} values obtained in challenge tests or storage trials with foods of interest. Other cardinal parameters values are kept constant when fitting Eq. 2 to μ_{max} values determined in well characterized products. This food/product-oriented approach will include the effect of food structure in the μ_{ref} value and this is appropriate at a cardinal parameter cannot easily be determined food structure.

1. For *L. monocytogenes* Eq. 2 was fitted to 41 μ_{max} values to obtain the values of μ_{ref}, T_{min} and P_{max} as reported in Table 2. Products of cold-smoked or marinated salmon and Greenland halibut were used for product calibration of the *L. monocytogenes* μ_{max}–model [11].
2. For psychrotolerant LAB Eq. 2 was fitted to 48 μ_{max} values determined in seafood [11] and to 96 μ_{max} values determined in seafood ($n = 30$) and meat products ($n = 66$) [16]. This resulted in the μ_{ref}, T_{min}, and P_{max} values reported in Table 2.

2.6 Secondary Lag Time Models

Prediction of lag time can be most important in the assessment and management of growth for bacterial pathogens in food. For microorganisms in a given physiological state is has been shown that the product of their maximum specific growth rate and lag time is constant ($\mu_{max} \cdot \lambda = k$) for a wide range of growth conditions. Consequently $\lambda = k/\mu_{max}$ or λ = relative lag time (RLT) · generation time = RLT·Ln(2)/μ_{max} [21]. Therefore, secondary lag time models have been developed from secondary growth rate models by estimation of RLT or k [21].

1. For *L. monocytogenes* an RLT value of 4.5 was estimated from data reported by Ross [22]. Later, studies of naturally contaminated lightly preserved seafood has confirmed an RLT value of 4.5 to result in realistic predictions and this RLT value is used in the *L. monocytogenes* growth model [2, 10].
2. Growth of psychrotolerant LAB showed no systematic and significant lag times in the studied seafood and meat products and an RLT value of 0 is used in the LAB growth model [16].

2.7 Secondary Models for Maximum Population Density

Maximum cell concentration (N_{max}) of bacterial pathogens when growing in food can be strongly influenced by the food microbiota (*see* Subheading 2.2; Fig. 5). However, N_{max} may also depend on storage conditions and product characteristics and a secondary model for this effect can be relevant.

1. In challenge tests without growth dampening from other groups of microorganisms, *L. monocytogenes* grew to high cell concentrations and N_{max} showed no clear relation to storage conditions and product characteristics. A constant log(N_{max}) value of 8.5 log (cfu/g) is used for the *L. monocytogenes* growth model [4].
2. With the exception of lumpfish roe with pH 5.5, psychrotolerant LAB grow to high concentrations of 6.8–9.2 log (cfu/g) and a constant log (N_{max}) value of 8.5 log (cfu/g) is used for the LAB growth model [16].

2.8 Evaluation and Validation of the Developed Models

As shown above many aspects can influence the performance of predictive food microbiology models. Therefore it is important to evaluate this model performance by comparison of observed and predicted responses in well characterized foods. These studies preferably should including naturally contaminated products. Observed and predicted responses can be compared by graphical methods and by using indices of model performance. For comparison of observed and predicted growth rates (or generation times) the use of bias- and accuracy factors has been suggested (Eqs. 9 and 10, [23]). The bias factor (B_f) indicates an average overestimation or underestimation of growth and the accuracy factor (A_f) is a measure of the average difference between observed and predicted μ_{max} values. As an example, a bias factor of 1.25 indicates that predicted growth on average is 25% faster than observed growth.

$$\text{Bias factor}\,(\mu_{max}) = 10^{\left(\sum \log(\mu_{max}\ \text{predicted}/\mu_{max}\ \text{observed})/n\right)} \quad (9)$$

$$\text{Accuracy factor}\,(\mu_{max}) = 10^{\left(\sum |\log(\mu_{max}\ \text{predicted}/\mu_{max}\ \text{observed})|/n\right)} \quad (10)$$

B_f values can be used to grade the performance of secondary μ_{max}–models. Ross [22] suggested limits of 0.95–1.11 for good; 0.87–0.95 or 1.11–1.43 for acceptable and <0.87 or >1.43 for unacceptable performance of models for microbial pathogens. For spoilage microorganisms B_f values within the range of 0.85 to 1.25 was suggested by Mejlholm and Dalgaard [16] to correspond to acceptable model performance. A_f values >1.5 can be an indication of systematic deviation between observed and predicted μ_{max} values and it is suggested to use graphical methods in combination with indices for model performance [16]. Growth boundary models can be evaluated by the % correct predictions of growth and no-growth responses, % fail-dangerous predictions (i.e., no-growth predicted when growth was actually observed), and % fail-safe predictions (i.e., growth predicted when no-growth was actually observed) [5]. The performance of growth models including the effect of interaction between groups on microorganisms can be evaluated by comparison of observed and predicted maximum population density (N_{max} values) for bacterial pathogens and by using the acceptable simulation zone (ASZ) method [7, 12]. The ASZ-method is also valuable for comparison of observed and predicted growth under variable environmental conditions including dynamic storage temperatures [12].

1. The *L. monocytogenes* μ_{max} model (Eq. 2, Table 2) was evaluated in an international validation study with 707 growth and 307 no-growth responses [5]. B_f/A_f values were 1.0/1.5 for meat products (n = 702) and 1.0/1.5 for seafood products (n = 193). For growth/no-growth responses the model had 89% correct predictions, 5% fail-dangerous predictions and 6% fail-safe-predictions. This showed the growth and growth

boundary model to be successfully validated (*see* **Note 3**). From the validation studies, the model's range of applicability for growth prediction was determined to include temperature (2–25 °C), atmosphere (0–100% CO_2), water phase salt (0.7–9.0%), pH (5.4–7.7), smoke components/phenol (0–20 ppm), nitrite (0–150 ppm in product), acetic acid (0–11,000 ppm in water phase), benzoic acid (0–1800 ppm in water phase), citric acid (0–6500 ppm in water phase), diacetate (0–3800 ppm in water phase), lactic acid (0–61,000 ppm in water phase), and sorbic acid (0–1300 in water phase).

2. The psychrotolerant LAB μ_{max}–model (Eq. 2, Table 2) was evaluated using 229 growth responses [16]. B_f/A_f values of 1.03/1.22 for seafood ($n = 99$) and of 1.08/1.27 for meat products ($n = 130$) showed this growth model to be successfully validated (*see* **Note 3**). The models range of applicability for growth prediction was determined to include temperature (0 to 25 °C), water phase salt (0.1 to 6.4), pH (5.3 to 7.7), equilibrium concentrations of CO_2 (0 to 100%), smoke components (0–21.2 ppm), nitrite (0–209 ppm in product), acetic acid (0–12,600 ppm in water phase), benzoic acid (0–1600 ppm in water phase), citric acid (0–7300 ppm in water phase), diacetate (0–3000 ppm in water phase), lactic acid (0–67,000 ppm in water phase), and sorbic acid (0–1600 in water phase). The LAB model is appropriate for VP and CO_2-enriched MAP products, which are also the most frequently used methods for distribution of many chilled foods.
3. The interaction model including the inhibiting effect of psychrotolerant LAB on growth of *L. monocytogenes* was evaluated by comparison of observed and predicted maximum population density (N_{max}) values. Data from 39 inoculated and 45 naturally contaminated seafood were used, and these products included cold-smoked or marinated salmon and Greenland halibut, raw and brined shrimps and crayfish as well as roe products [1, 2, 7, 10, 11]. N_{max} values for *L. monocytogenes* was well predicted (Fig. 2) with average observed and predicted $\log(N_{max})$ values of, respectively, 4.44 log cfu/g and 4.15 log cfu/g for inoculated products and, respectively, 0.53 log cfu/g and 0.53 log cfu/g for naturally contaminated products. Microbial interaction has a strong effect on growth of *L. monocytogenes* in naturally contaminated seafood and this effect was well predicted by the developed model for simultaneous growth of psychrotolerant LAB and the bacterial pathogen (Fig. 2). To further explain this effect it can be mentioned that average N_{max} values were predicted to be 14 times too high if the effect of organic acids was not taken into account. However, without the effect of both organic acids and LAB the

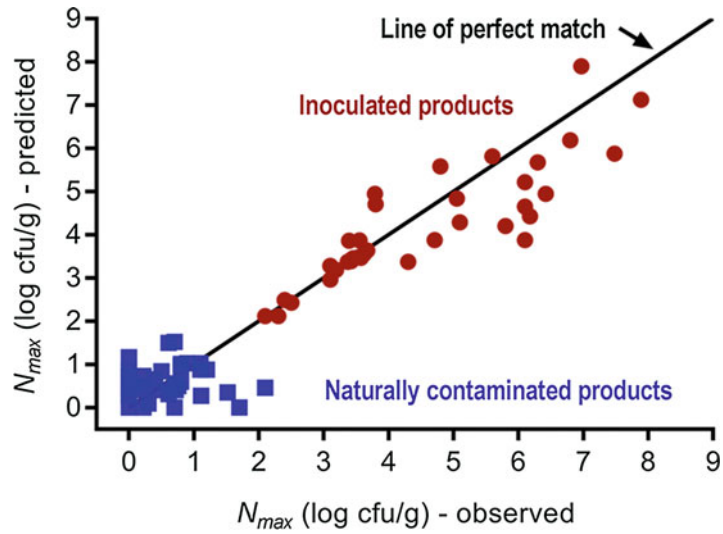

Fig. 2 Comparison of observed and predicted maximum population density (N_{max}) values (log cfu/g) for *Listeria monocytogenes* in inoculated (circles) and naturally contaminated (squares) seafood products

predicted average N_{max} values was 9500 times too high for the studied naturally contaminated seafood products.

2.9 Practical Use of Models and Application Software

In Europe, it is the responsibility of food business operators to document that *L. monocytogenes* does not growth to more than 100 cfu/g during shelf-life of ready-to-eat products [24]. This represents considerable challenges for various RTE-foods including cold-smoked salmon and other lightly preserved seafood where prevalence of this bacterial pathogen can be as high at 3–8% [25]. The examples below show how the developed models and software can contribute to overcome these challenges:

1. The developed models for *L. monocytogenes*, psychrotolerant LAB and their simultaneous growth have been included in the Food Spoilage and Safety Predictor (FSSP) software. This facilitates practical application of the models as FSSP is available for free (http://fssp.food.dtu.dk) and includes modules to calculate required model input to obtain predictions of growth and growth boundary (*see* **Note 4**).
2. As an example of model application, for vacuum-packed cold-smoked salmon (CSS) growth of *L. monocytogenes* corresponding to a 100-fold increase in cell-concentration was predicted during 25–26 days of storage at 5 °C (Fig. 3). Furthermore, FSSP predicted a reformulation of CSS by addition of acetic acid (2000 ppm in water phase of product) to prevent growth of *L. monocytogenes* at 5 °C as shown by blue

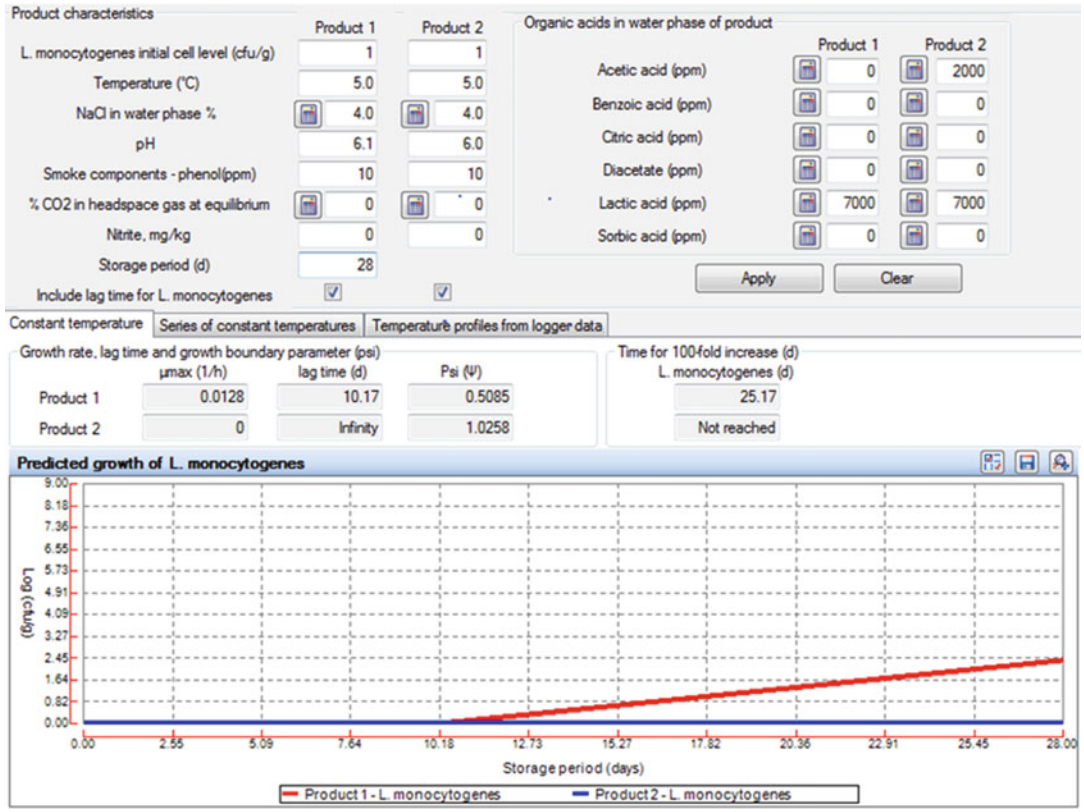

Fig. 3 Predicted growth of *Listeria monocytogenes* by the FSSP software in vacuum packed cold-smoked salmon without (Product 1) and with (Product 2) addition of acetic acid to the product

curve in the graph and by the ψ-value of just above 1.0 (*see* **Notes 5** and 7).

3. The application of predictive food microbiology models for documentation of food safety as shown above is included in the EU-regulation [24]. In 2013 the Danish Veterinary and Food Administration (Competent authority) specified that samples from five separate batches had to be analyzed when the developed *L. monocytogenes* model in FSSP was used to document safety of lightly preserved seafood. Samples should be analyzed for dry matter, pH, NaCl, food preservatives (including naturally occurring lactate) and smoke components as relevant. The least preserved of the five samples then determine if a product is categorized as stabilized against growth of *L. monocytogenes* (Cat. 1.3) or otherwise (Cat. 1.2) according to the EU-regulation [24] (*see* https://www.foedevarestyrelsen.dk/SiteCollectionDocuments/Foder%20og%20foedevaresikkerhed/Alt-om-listeria/FISK-listeria-planche.pdf).
4. FSSP allows growth of *L. monocytogenes* to be predicted at dynamic temperature conditions. For CSS with acetic acid as

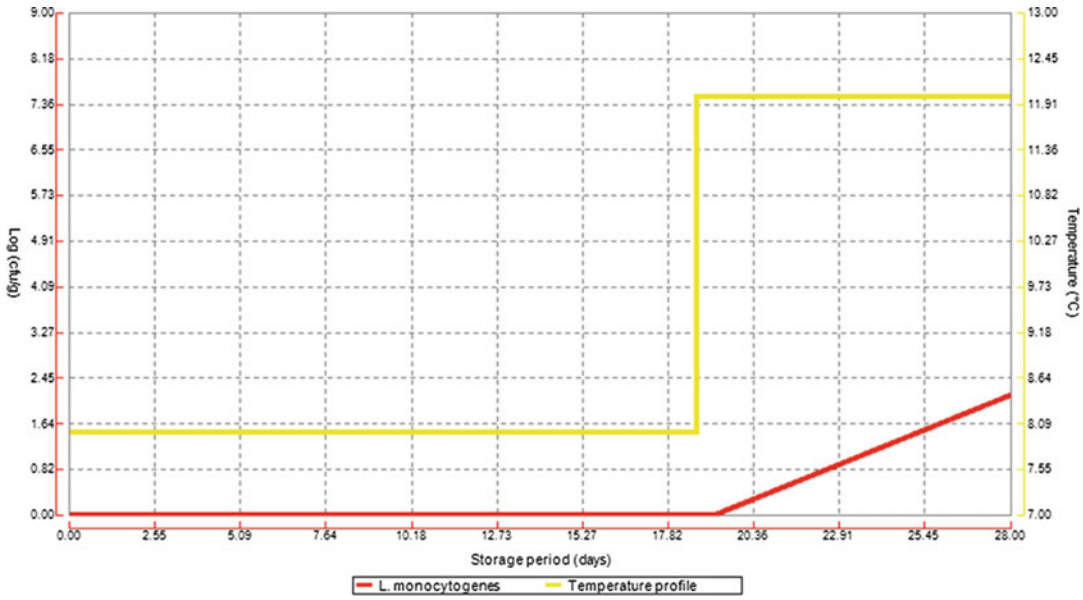

Fig. 4 Effect of simple temperature profile as predicted by the FSSP software for growth of *Listeria monocytogenes* in vacuum packed cold-smoked salmon

shown in Fig. 3 (Product 2, blue line) a series of constant temperatures with 8 °C during two-thirds of the shelf-life (448 h) followed by 12 °C during one-third of the shelf-life (224 h) resulted in a predicted 100-fold increase in cell-concentration during 27–28 days of storage (Fig. 4). For food products in distribution, temperature profiles from data loggers can contribute to establish realistic temperature storage conditions. The FSSP software can then read these temperature profiles and predictions can be used to establish product characteristic resulting in a sufficient degree of growth inhibition for realistic temperature storage conditions (*see* **Note 6**).

5. LAB can have a significant inhibiting effect on growth of *L. monocytogenes*. For CSS without acetic acid as shown in Fig. 3 (Product 1, red line) FSSP predicts that and initial LAB concentration of 1000 cfu/g will prevent further growth of *L. monocytogenes* after about 18 days of storage at 5 °C (Fig. 5). The predicted increase in the cell concentration of *L. monocytogenes* will be limited by LAB to about sevenfold, corresponding to 0.8 log(cfu/g), within the storage period of 28 days. Thus, LAB reduced the predicted growth of *L. monocytogenes* after 28 days of storage from 2.4 to 0.8 log cfu/g (Fig. 5).
6. The growth boundary of *L. monocytogenes* and the distance to the growth boundary, as expressed by the ψ-value, can be predicted by FSSP (Fig. 6). These predictions are important

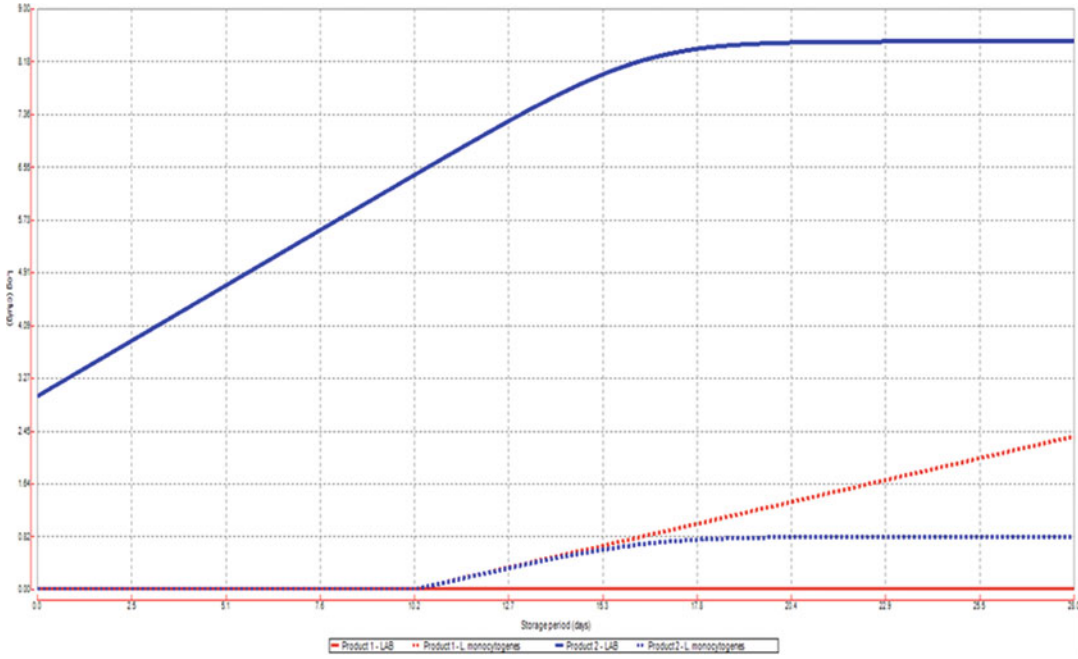

Fig. 5 Effect of psychrotolerant LAB (0 or 1000 cfu/g) on growth of *Listeria monocytogenes* in vacuum packed cold-smoked as predicted by the FSSP software

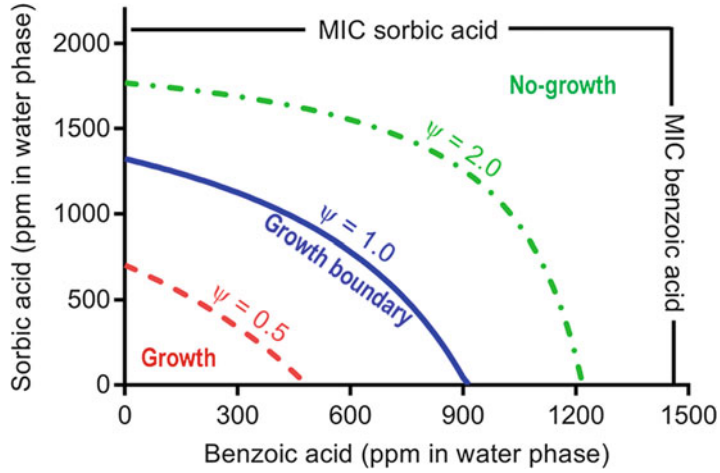

Fig. 6 Growth boundary (ψ value = 1.0) and boundary conditions of Listeria monocytogenes growth in modified atmosphere packed brined shrimps at 5 °C and with 3.0%WPS, pH 5.7, 25% CO_2 in the headspace gas at equilibrium and 6000 ppm citric acid in the water phase

as lightly preserved food with long shelf-life need to be formulated to prevent growth of the pathogen (ψ-value >1) and at the same time with a sufficient distance to the growth boundary so that variability in product characteristics does

not lead to unacceptable growth of *L. monocytogenes* (*see* **Note 7**). Boundary conditions in Fig. 6 were predicted for modified atmosphere packing brined shrimps with benzoic and sorbic acids. The product had 3.0%WPS, pH 5.7, 25% CO_2 in the headspace gas at equilibrium and 6000 ppm citric acid in the water phase. The predicted growth boundary (ψ-value = 1.0) is far from the MIC -values of both benzoic and sorbic acids (Fig. 6). This illustrates a pronounced effect of interaction between environmental parameters (Eqs. 3–5) on growth inhibition close to the growth boundary. For lightly preserved foods where several environmental factors contribute to reduce growth of microorganisms interaction between these environmental factors will often be important. The Le Marc -approach is an efficient way to model the effect of interaction between environmental factors (Eqs. 3–5) and to predict growth boundary and boundary conditions as shown in Fig. 6.

3 Notes

1. The primary and secondary growth models described in this chapter have been selected for their relative simplicity compared to complexity of the foods where growth is predicted. Numerous other and other more complex primary and secondary growth models are available [21, 26].
2. For acetic, benzoic, citric and sorbic acids the obtained MIC values for psychrotolerant LAB resulted in prediction of μ_{max} values in seafood and meat products that were too low compared to validation studies. The broth experiments were repeated twice with comparable results. As an alternative, these MIC values were determined from μ_{max} values of *L. sakei* in challenge tests with brined and drained shrimp including different concentrations of organic acids. These MIC values were 2–3 times higher than those obtained using APT broth, and importantly they resulted in a good/acceptable performance of the new model [16].
3. Different validation studies have shown that data from more than about 20 well-characterized products is need for bias- and accuracy-factor values to become stable and useful for evaluation of the performance of μ_{max} models [5, 16].
4. To make accurate predictions a model has to use input (storage conditions and product characteristics) that is determined in the same way as used when the model was developed and validated. To facilitate the determination of model input, FSSP has built-in modules to calculate water-phase concentrations of NaCl and organic acids from their total concentration and from the dry matter content of products. Importantly,

model input to FSSP is water-phase NaCl (water phase salt, WPS) rather than a_w. Thus, WPS must be determined for products where predictions are needed. Measured a_w values of products can reflect the effect of compounds other than NaCl and therefore lead to prediction of too little growth if the measured a_w values are used directly in Eq. 2. FSSP calculates a_w values from % WPS ($a_w = 1–0.0052471 \cdot \%WPS-0.00012206 \cdot \%WPS^2$). pH is measured in blended food samples dilute in water. Model input for smoke intensity of smoked product is measured as the phenol concentration. A particular method is used relying on ethanol extraction of phenolic compounds and quantification by spectroscopy after reaction with amino-4-antipyrin [27]. For modified atmosphere packed products the model input (% CO_2 in headspace gas at equilibrium) can be measured by a gas analyzer about 1 day after packaging when CO_2 has dissolved into the water-phase of the product. FSSP can also calculate this model input from initial % CO_2 in the headspace gas at packaging, initial gas/product ratio and storage temperature. Nitrite is determined as the initial concentration in products (ppm). Model input for organic acids is ppm in water-phase of products.

5. Addition of acetic acid, for example as sodium acetate or sodium diacetate (E262), at a concentration of 1000–2500 ppm in the water-phase of CSS, and other lightly preserved seafood, will slightly reduce pH by 0.1–0.2 units. The combined effect of acetic acid and other product characteristic on growth inhibition of *L. monocytogenes* is substantial and important for safety of different lightly preserved foods. Importantly, the developed *L. monocytogenes* model in FSSP allows food business operators to formulate or reformulated products by taking into account various product characteristics. This facilitate product development as many different combinations of environmental factors, including sodium/salt reduction, can result is a desired degree of growth inhibition and predictions can be obtained rapidly in comparison with product studies including challenge tests. A lag-phase is included when predicting *L. monocytogenes* growth in lightly preserved seafood as this has been observed for naturally contaminated products [2].
6. Temperature loggers use numerous formats for temperature profile data. To read data from different loggers FSSP allows temperature profiles to be entered by copy and paste from spreadsheets.
7. ψ-values and boundary conditions for *L. monocytogenes* growth in lightly preserved food deserves further study in relation to variability of product characteristics. However, available information suggests (1) ψ-values of <1 can be acceptable for product with shelf-life of <2–3 week; (2) ψ-values of 1–2 can

be acceptable for product with shelf-life of 3–5 weeks and (3) ψ-values of >2 are needed for product with shelf-life of >5 weeks [4, 5].

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