Food Microbiology 46 (2015) 1–14

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

# Modelling and predicting the simultaneous growth of *Listeria monocytogenes* and psychrotolerant lactic acid bacteria in processed seafood and mayonnaise-based seafood salads

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ARTICLE INFO

*Article history:*
Received 16 December 2013
Received in revised form
16 June 2014
Accepted 4 July 2014
Available online 15 July 2014

*Keywords:*
Predictive model
Microbial interaction
Jameson effect
Lactobacillus
Risk assessment
Risk management
Organic acids

ABSTRACT

A new combined model for *Listeria monocytogenes* and psychrotolerant *Lactobacillus* spp. was constructed and evaluated for processed seafood and mayonnaise-based seafood salads. The new model was constructed by combining existing cardinal parameter models for *L. monocytogenes* and *Lactobacillus* spp. using the classical Jameson effect to model microbial interaction. Maximum population density (MPD) values of *L. monocytogenes* were accurately predicted in processed seafood with a known initial cell concentration of *Lactobacillus* spp. For these experiments, average MPD values of 4.5 and 4.3 log (cfu/g) were observed and predicted, respectively for *L. monocytogenes*. In seafood salads, growth of *L. monocytogenes* continued at a reduced growth rate after *Lactobacillus sakei* had reached their MPD. This growth pattern was successfully described by an expanded version of the classical Jameson effect model, but only for products with pH of 6.0 or higher. For seafood salads with pH below 6.0 the performance of the new model was unacceptable, primarily due to prediction of no-growth by *L. monocytogenes* when growth was actually observed. Within its range of applicability the new model can be valuable for risk assessment and risk management of processed seafood as well as for evaluating the compliance of products with the EU regulation for ready-to-eat foods.



## 1. Introduction

Over the last couple of decades, numerous mathematical models have been developed to predict growth responses of pathogenic and spoilage microorganisms in different types of food. These models are important as they facilitate the assessment and management of microbial food safety and quality. Some predictive models have been included in user-friendly software and are widely used by food processors and regulatory authorities (McMeekin et al., 2006). As one example, the Seafood Spoilage and Safety Predictor (SSSP) software has been recommended by the Danish Veterinary and Food Administration (DVFA) as a means to predict growth of *Listeria monocytogenes* and to document the compliance of ready-to-eat foods with the EU-regulation (EC, 2005; DVFA, 2013). SSSP includes a growth and growth boundary model for *L. monocytogenes* that takes into account the effect of 12 environmental parameters (Mejlholm and Dalgaard, 2009). This model has been validated and is increasingly used for different types of food with various preserving parameters (Mejlholm et al., 2010). However, further studies are needed to determine its range of applicability with respect to other food types and preserving parameters. Previously, modelling the effect of microbial interaction between *L. monocytogenes* and lactic acid bacteria (LAB) was determined to be important in order to accurately predict the maximum population density (MPD) of the pathogen e.g. for cold-smoked and marinated fish products (Giménez and Dalgaard, 2004; Mejlholm and Dalgaard, 2007a; Vermeulen et al., 2011). These predictions relied on (i) the assumption of the Jameson effect i.e. that *L. monocytogenes* stopped growing when LAB reached their MPD and on (ii) accurate predictions of growth responses of both *L. monocytogenes* and LAB. Existing predictive models for growth of *L. monocytogenes* and psychrotolerant LAB have been successfully validated for wide ranges of 12 preserving parameters and including products with benzoic, citric and/or sorbic acids (Mejlholm and Dalgaard, 2009, 2013). However, the ability of these models to accurately predict microbial interaction in chilled products including these organic acids remains to be documented and

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http://dx.doi.org/10.1016/j.fm.2014.07.005
0740-0020/

this is important e.g. for brined shell-fish, roe-products and seafood salads, including mayonnaise-based products, where benzoic, citric and/or sorbic acids often are used as preserving parameters (Mejlholm et al., 2012; Skalina and Nikolajeva, 2010; Vermeulen et al., 2007b).

Several studies have shown the presence and growth potential of *L. monocytogenes* in mayonnaise-based salads, with the highest prevalence of the pathogen being observed for products containing processed seafood (Coillie et al., 2004; Di Pinto et al., 2010; Gombas et al., 2003; Hartemink and Georgsson, 1991; Levine et al., 2001; Uyttendaele et al., 2009). With respect to microbial spoilage of these products, LAB has often been identified as the dominating microbiota at the time of sensory rejection, together with yeast and moulds (Manios et al., 2009; Smittle and Flowers, 1982). Some mathematical models for growth responses of *L. monocytogenes* or LAB in mayonnaise-based salads and related products are available (Gysemans et al., 2007; Hwang and Tamplin, 2005; IFST, 1993; Manios et al., 2009, 2014; Vermeulen et al., 2007a,b). These models, however, do not include the effect of benzoic, citric and sorbic acids or the effect of microbial interaction between *L. monocytogenes* and LAB. In addition, several of the models are developed exclusively for ambient temperature storage and further studies of chilled mayonnaise-based seafood salads are needed.

The objective of the present study was to model and predict the simultaneous growth of *L. monocytogenes* and psychrotolerant LAB in processed and chilled seafood and mayonnaise-based seafood salads with different combinations of preservatives including benzoic, citric and/or sorbic acids. Existing growth and growth boundary models for *L. monocytogenes* and psychrotolerant *Lactobacillus* spp. including the individual as well as the interactive effect of 12 environmental parameters (Mejlholm and Dalgaard, 2009, 2013) were combined to take into account the effect of microbial interaction between the two types of microorganisms. The performance of the new combined model was evaluated by comparison of observed and predicted growth of *L. monocytogenes* and *Lactobacillus sakei* in 30 challenge tests with brined shrimp and mayonnaise-based shrimp salad including different combinations of organic acids as preservatives, and stored at both dynamic and constant chill storage temperatures. Literature data ($n = 82$) for kinetics of *L. monocytogenes* and LAB in different types of seafood was included to strengthen the validation of the new combined model.

## 2. Material and methods

### 2.1. Development of the new combined model for L. monocytogenes and psychrotolerant Lactobacillus spp.

Cardinal parameter secondary models for the growth rate and the growth boundary of *L. monocytogenes* and psychrotolerant *Lactobacillus* spp. (Mejlholm and Dalgaard, 2009, 2013) were combined by using the expanded and differential form of the logistic model (Equation (1)) to model the effect of microbial interactions as previously described (Giménez and Dalgaard, 2004; Mejlholm and Dalgaard, 2007a). Both secondary models include the effect of temperature, NaCl/water activity, pH, smoke components (phenol), $CO_2$, nitrite, acetic acid, benzoic acid, citric acid, diacetate, lactic acid and sorbic acid as well as their interactive effects. The present study concerns chilled products and unless otherwise specifically stated then predictions for LAB and *Lactobacillus* spp. refer to psychrotolerant species.

An expanded version of the differential form of the logistic model was used to model microbial interaction between *L. monocytogenes* and LAB (Equation (1)) (Giménez and Dalgaard, 2004; Møller et al., 2013). This model is based on the assumption that *L. monocytogenes* and LAB inhibit each other to the same extent as they inhibit their own growth. However, due to higher concentrations and faster growth of LAB, the inhibiting effect of *L. monocytogenes* on LAB was not evaluated in the present study.

$$\begin{cases} t < t_{\mathrm{lag-Lm}}, & \dfrac{\mathrm{dLm}/\mathrm{d}t}{\mathrm{Lm}_t} = 0 \\ t \geq t_{\mathrm{lag-Lm}}, & \dfrac{\mathrm{dLm}/\mathrm{d}t}{\mathrm{Lm}_t} = \mu_{\max \mathrm{Lm}} \times \left(1 - \dfrac{\mathrm{Lm}_t}{\mathrm{Lm}_{\max}}\right) \times \left(1 - \dfrac{\gamma \times \mathrm{LAB}_t}{\mathrm{LAB}_{\max}}\right) \\ t < t_{\mathrm{lag-LAB}}, & \dfrac{\mathrm{dLAB}/\mathrm{d}t}{\mathrm{LAB}_t} = 0 \\ t \geq t_{\mathrm{lag-LAB}}, & \dfrac{\mathrm{dLAB}/\mathrm{d}t}{\mathrm{LAB}_t} = \mu_{\max \mathrm{LAB}} \times \left(1 - \dfrac{\mathrm{LAB}_t}{\mathrm{LAB}_{\max}}\right) \end{cases} \quad (1)$$

where Lm and LAB, both >0 cfu/g, signify concentrations of *L. monocytogenes* and LAB, respectively, and $\gamma$ is a competition factor that allows the predicted cell concentration of *L. monocytogenes* to increase ($\gamma < 1$) or decrease ($\gamma > 1$) after the cell concentration of LAB has reached their maximum population density (MPD) value ($\mathrm{LAB}_{\max}$) (Møller et al., 2013). Growth rates of *L. monocytogenes* ($\mu_{\max\ \mathrm{Lm}}$) and LAB ($\mu_{\max\ \mathrm{LAB}}$) were obtained from the models of Mejlholm and Dalgaard (2009, 2013).

### 2.2. Bacterial strains and preculture conditions

Four isolates of *L. sakei* (LA-5, A1, MAP23-1 and F46-4) (LAB mix) and four isolates of *L. monocytogenes* (94-203D, 95-54A, 95-442A, 94-167B) (Lm mix) from processed seafood (e.g. brined and drained MAP shrimp and seafood salad) (Giménez and Dalgaard, 2004; Jørgensen and Huss, 1998; Mejlholm et al., 2008; Mejlholm and Dalgaard, 2007a) were used for inoculation of challenge tests. Initially, each isolate was transferred from a −80 °C culture collection at DTU Food and grown at 25 °C for 24 h using All Purpose Tween (APT) broth (Difco 265510, Becton, Dickinson and Company, Le Point de Claix, France) for *L. sakei* and Brain Heart Infusion (BHI) broth (Oxoid CM1135, Basingstoke, Hampshire, England) for *L. monocytogenes*. Then isolates were precultured at 8 °C for 2–3 days in fresh medium of the same type with pH 6.0 and 2% NaCl to simulate the conditions of many processed seafood. Precultures were harvested in late exponential growth phase, defined as a relative change in absorbance of 0.05–0.2 at 540 nm (Novaspec II, Pharmacia Biotech, Allerød, Denmark). As needed, precultures were diluted in 0.85% NaCl prior to inoculation of the products used in the various experiments.

### 2.3. Evaluation of the new combined model for L. monocytogenes and psychrotolerant Lactobacillus spp.

112 sets of environmental conditions and corresponding growth responses of *L. monocytogenes* and LAB in processed seafood and mayonnaise-based seafood salads were used to evaluate the performance of the new combined Lm–LAB model (Tables 1 and 2). A total of 30 data sets from brined and drained shrimp ($n = 11$) and mayonnaise-based shrimp salad ($n = 19$) were generated in the present study (Table 1). In addition, data from 82 experiments with processed seafood and mayonnaise-based seafood salads were obtained from the literature (Table 2). 57 experiments were inoculated with *L. monocytogenes* and LAB, whereas for the remaining 55 experiments the products were inoculated exclusively with the pathogen (Tables 1 and 2).

For literature data, growth responses of *L. monocytogenes* and LAB were described by (i) the maximum population density (MPD,