A mathematical model to predict the antilisteria bioprotective effect of *Latilactobacillus sakei* CTC494 in vacuum packaged cooked ham

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Abstract

Biopreservation is a strategy that has been extensively covered by the scientific literature from a variety of perspectives. However, the development of quantitative modelling approaches has received little attention, despite the usefulness of these tools for the food industry to assess the performance and to set the optimal application conditions. The objective of this study was to evaluate and model the interaction between the antilisteria strain *Latilactobacillus sakei* CTC494 (sakacin K producer) and *Listeria monocytogenes* in vacuum-packaged sliced cooked ham. Cooked ham was sliced under aseptic conditions and inoculated with *L. monocytogenes* CTC1034 and/or *L. sakei* CTC494 in monoculture and coculture at 10:10, 10:10^3 and 10:10^5 cfu/g ratios of pathogen:bioprotective cultures. Samples were vacuum packaged and stored at isothermal temperature (2, 5, 10 and 15 ºC). The growth of the two bacteria was monitored by plate counting. The Logistic growth model was applied to estimate the growth kinetic parameters (N_0, λ, μ_{max}, N_{max}). The effect of storage temperature was modelled using the hyperbola (λ) and Ratkowsky (μ_{max}) models. The simple Jameson-effect model, its modifications including the N_{cri} and the interaction γ factor, and the predator-prey Lotka Volterra model were used to characterize the interaction between both microorganisms. Two additional experiments at non-isothermal temperature conditions were also carried out to assess the predictive performance of the developed models through the Acceptable Simulation Zone (ASZ) approach. In monoculture conditions, *L. monocytogenes* and *L. sakei* CTC494 grew at all temperatures. In coculture conditions, *L. sakei* CTC494 had an inhibitory effect on *L. monocytogenes* by lowering the N_{max}, especially with increasing levels of *L. sakei* CTC494 and lowering the storage temperature. At the lowest temperature (2 ºC) *L. sakei* CTC494 was able to completely inhibit the growth of *L. monocytogenes* when added at a concentration 3 and 5 Log higher than that of the pathogen. The inhibitory effect of the *L. sakei* CTC494 against *L. monocytogenes* was properly characterized and modelled using the modified Jameson-effect with interaction γ factor model. The developed interaction model was tested under non-isothermal conditions, resulting in ASZ values ≥ 83%. This study shows the potential of *L. sakei* CTC494 in the biopreservation of vacuum-packaged cooked ham against *L. monocytogenes.*

The developed interaction model can be useful for the industry as a risk management tool to assess and set biopreservation strategies for the control of *L. monocytogenes* in cooked ham.

Keywords: *Listeria monocytogenes*, Lactic acid bacteria, microbial interaction models, bacteriocins, safe shelf-life, *Lactobacillus sakei*

1. Introduction

Listeria monocytogenes is one of the relevant pathogens for ready-to-eat (RTE) cooked meat products due to the risk of listeriosis. Though it shows a relatively low morbidity, listeriosis severity is high, showing the highest hospitality and case-fatality rates among all foodborne bacteria, in the European Union (EU) accounting for 50% up to 67% of deaths depending on the year (ECDC & EFSA 2015; ECDC & EFSA 2019). Despite increasing awareness and the application of control measures focused on *L. monocytogenes* in food, listeriosis keeps a statistically significant increasing trend since 2009 (ECDC/EFSA, 2019). In 2019, the biggest listeriosis outbreak in EU occurred in Spain linked to the consumption of cooked meat products produced by a single manufacturer (WHO, 2019). Major listeriosis outbreaks occurring worldwide have also been linked to cooked meat products, e.g. Canada, 1985 (Maple Leaf Foods), USA (Farber et al., 2007), South Africa, 2017 (Thomas et al., 2020).

Food biopreservation consists of the use of microorganisms and/or their metabolites as an innocuous and ecological approach to extend the safe shelf-life of perishable products with minimal impact on the sensory characteristics. In the last decades, the interest towards a variety of microorganisms, particularly lactic acid bacteria (LAB), as bioprotective cultures to inhibit pathogenic bacteria, mainly *L. monocytogenes*, in meat products has been explored for long time by the scientific community (Amézquita & Brashears, 2002; Andersen, 1995; Bredholt et al., 1999; Bredholt et al., 2001; Budde et al., 2003; Danielski et al., 2020; Devlieghere et al., 2001; Hugas et al., 1998; Lucke, 2000; Mataragas et al., 2003; Rivas et al., 2014; Vermeiren et al., 2005). The psychotrophic nature of some LAB makes these bioprotective cultures a versatile strategy to control the growth of *L. monocytogenes* in foods, including chilled foods with extended shelf-life (Aymerich et al., 2006). Within LAB species, the use of bacteriocin producing strains with antilisteria activity can inhibit the growth and even compromise the viability of *L. monocytogenes* in meat products. Moreover, the production of bacteriocins in situ by the bioprotective cultures allow to avoid a possible loss of effectiveness of the bacteriocin when applicated (semi)purified in products, which in turn are not authorized as food preservatives in most of the countries (Aymerich et al., 2006). Instead, food cultures consisting

of microbial species with Qualified Presumption of Safety (QPS) status according to the European Food Safety Authority (EFSA) or Generally Recognised As Safe (GRAS) to U.S. Food and Drug Administration can be used as food ingredients (Laulund et al., (2017). All these characteristics pointed out that LAB bioprotective cultures and their metabolites can be a feasible option to be considered by food manufacturers to extend the safe shelf-life of their perishable products, while complying with the regulated *L. monocytogenes* microbiological criteria applicable to RTE food stating a maximum acceptable limit of 100 cfu/g during the shelf-life of the food (e.g. Codex, 2007; European Commission, 2005; Health Canada, 2011). Despite biopreservation through LAB has been extensively covered by the scientific literature from a variety of perspectives (e.g. potential technology applications, molecular mechanisms of action, etc.), the development of quantitative modelling approaches to address biopreservation has received much less attention so far. Predictive microbiology, also known as quantitative microbial ecology, is a useful approach to characterize and quantify the behaviour of microorganisms in food as a function of extrinsic and intrinsic factors through the use of primary and secondary models (Buchanan et al., 1997). The interaction between microbial groups (i.e. implicit factors) needs a bit more complex modelling approach (Cadavez et al., 2019). The competitive or antagonistic interactions between the bioprotective LAB culture and *L. monocytogenes* reported objective data on the antilisteria effect of the LAB cultures and on the consequent extension of the safe shelf-life. Within this context, interaction models that describe the simultaneous growth of each microorganism, taking into account the limited nutrient availability and the production of metabolites (lactic and acetic acids and bacteriocins among others) by LAB can be used to quantify the interaction of LAB and *L. monocytogenes* in real food matrices. Approaches based on the Jameson effect (Cornu et al., 2011) rely on the simultaneous deceleration of all microbial populations when the dominating microorganism reaches the stationary growth phase and inhibits the other to the same extend as they inhibit their own growth. In practice, this means that the maximum population density of *L. monocytogenes* is restricted by the growth of LAB. Jameson effect models were originally proposed to simulate the growth of two populations in mix cultures based on growth parameters

predicted from secondary growth models or estimated in pure cultures. In the case of incorporating, in the model, interaction-related parameters, the use of arbitrary values for these parameters may lead to discrepancies when predictions are compared with actual observed data (Cornu et al., 2011). Jameson effect models can also be used to fit growth curves with non-linear regression tools (Cornu et al., 2011), which provides adjusted values of the interaction related parameters leading to satisfactory predictive performance of the developed models (Costa et al., 2019). Jameson effect models are widespread and have been applied for describing microbial interaction between background microbiota and pathogens in milk, cheese, vegetables, fish and meat (Coleman et al., 2003; Giménez & Dalgaard, 2004; Guillier et al., 2008; Østergaard et al., 2014; Ross et al., 2000). Some modifications of this model have been proposed allowing to quantify the growth of the pathogen after the dominant (spoilage) microbial group reaches its maximum population (Giménez & Dalgaard, 2004) or to characterize the critical concentration of the dominant microorganism at which the pathogen stops growing (Le Marc et al., 2009). An empirical variant of the Jameson-effect model includes the use of the standard primary model for the population of interest and build a secondary model on the maximum population density parameter as a function of relevant environmental parameters (Cornu et al., 2011). A different approach is the one behind the Lotka-Volterra model, also known as a predator-prey model. Its underlying mechanism is the competition for a common substrate which allow to describe the dynamics of two interacting bacterial populations through competition factors that describe the reduction of the growth rate of a given population (Cornu et al., 2011; Powell et al., 2004; Valenti et al., 2013; Vereecken et al., 2000). Interaction models have been scarcely applied to quantitatively characterize the performance of bioprotective bacteria with specific antagonistic activities (e.g. through the bacteriocin production), which are intentionally added to the food at usually higher levels than those of the naturally present background (spoilage) microbiota.

In this framework, the objective of the present study was to quantitatively assess the effect of the sakacin-producing bioprotective strain *Latilactobacillus sakei* CTC494, previously *Lactobacillus sakei* (Zheng et al., 2020), on the inhibition of *L. monocytogenes* growth in

vacuum packaged sliced cooked ham during refrigerated storage. This strain was previously reported to have antilisteria activity through the production of sakacin K (Hugas et al., 1993). The final purpose was to apply and validate a microbial interaction model to describe the interaction between both microorganisms at isothermal and non-isothermal conditions in order to provide food business operators with a versatile tool for the assessment and proper implementation of biopreservation for ensuring the safety of cooked ham during shelf-life.

2. Material and methods

2.1 Bacterial strains

The strain of *L. monocytogenes* used in the present study was the meat isolate CTC1034 (serotype 4b) from IRTA culture collection, previously used in our studies dealing with preservation of meat products (Bover-Cid et al., 2011, Bover-Cid et al., 2015; Bover-Cid et al., 2019; Hereu et al., 2012a; Hereu et al., 2012b; Hereu et al., 2014). As a bioprotective culture, the bacteriocin-producing *L. sakei* CTC494 strain was used. Sakacin K is the bacteriocin produced by this strain, which has been shown to inhibit the growth of spoilage bacteria and *Listeria* spp. (Hugas et al., 1993), including *L. monocytogenes* in different types of food (Aymerich et al., 2019; Costa et al., 2019; Hugas et al., 1995). The production of bacteriocin by the *L. sakei* CTC494 was confirmed the whole range of temperatures tested in the present study. De Man Rogosa and Sharpe broth medium (MRS, Oxoid, UK) was used to store at -80 ºC the *L. sakei* CTC494 strain stock culture while for the *L. monocytogenes* CTC1034 strain, the Brain Heart Infusion (BHI) broth (Beckon Dickinson, Sparks, Md., USA) was used. Both mediums were supplemented with 20% glycerol as cryoprotectant.

2.2 Cooked ham manufacture, sample preparation and inoculation

Cooked ham was manufactured using shoulder pork meat and the following ingredients (g/kg): water, 120; salt, 20.7; sodium tripolyphosphate, 5.8; dextrose, 5.8; carrageenan, 2.3; sodium ascorbate, 0.6; and sodium nitrite 0.1. Meat was minced in a cutter to a particle size of 6 mm. Ingredients were homogenized in a mixer for 30 min, stuffed into an impermeable plastic

casing, and cooked in an oven at 68 °C for 5 h, the products reaching the core temperature of 65 °C. The manufactured cooked ham was composed of 1.60% fat, 19.56% protein, 75.64% moisture, 0.64% collagen, 2.72% salt (NaCl), 0.7% of lactic acid (endogenous) and with a pH 6.07 ± 0.03 and a_w 0.978 ± 0.001, in agreement with previous works (Bover-Cid et al., 2019). Cooked ham was sliced in the laboratory under aseptic conditions. Slices were spiked with either the *L. monocytogenes* CTC1034 strain or/and *L. sakei* CTC494 with 1% (v/w) of the corresponding culture diluted in physiological saline water (PSW, 0.85% w/v NaCl) to set up the required initial inoculum concentration. For monoculture experiments, both the pathogen and the bioprotective LAB were inoculated at ca. 10 cfu/g. For the coculture experiments, the pathogen concentration was also set up to ca. 10 cfu/g and for the bioprotective LAB three different initial concentrations were studied: 10, 10^3 and 10^5 cfu/g, respectively; making the concentration of bioprotective strain similar, 100-fold and 10000-fold higher than that of the pathogen, respectively. Therefore, the three initial concentration pathogen:bioprotective cultures studied corresponded to ratios 10:10, 10:10^3 and 10:10^5cfu/g. After inoculation, samples were vacuum packaged (EV-15-2-CD; Tecnotrip, Terrassa, Spain) in PET/PE bags (oxygen permeability < 50 cm^3/m^2/24 h and low water vapour permeability < 15 mg/m^2/24 h; Sacoliva S.L., Barcelona, Spain). Samples of each inoculation ratio were randomly distributed in four groups to be stored at 2, 5, 10 and 15 ºC, respectively. The storage time ranged from 15 days (at the highest temperature) to 150 days (at the lowest temperature).

2.3 Monitoring bacterial concentrations along the storage time

Each sample (25 - 30 g) was homogenized 1/10 in a bag Blender Smasher® (bioMérieux, Marcy-l'Étoile, France) and 10-fold serially diluted in physiological saline solution (0.85% NaCl and 0.1% Bacto Peptone). Enumeration of *L. monocytogenes* was performed on the CHROMagar™ Listeria chromogenic media (CHROMagar, Paris, France) incubated at 37 °C for 48 h. To achieve a quantification limit of 2 cfu/g, 5 mL of the 1/10 diluted homogenate was pour plated into plates with a diameter of 14 cm (Hunt et al., 2017). For samples with expected concentration of *L. monocytogenes* below this quantification limit, the presence/absence of the

pathogen was investigated by enrichment of 25 g-samples in 225 mL tryptic soy broth (Becton Dickinson) supplemented with 0.6% yeast extract (TSBYE) and incubated for 48 h at 37 °C. After enrichment, the presence of *L. monocytogenes* was detected by plating on CHROMagar™ Listeria. Enumeration of *L. sakei* CTC494 was conducted on de MRS agar media incubated at 30 ºC for 72 h under anaerobiosis conditions. Endogenous LAB levels in cooked ham non-inoculated with bioprotective culture were below the limit of detection (10 cfu/g).

2.4. Primary growth modelling

The primary kinetic growth parameters of both *L. monocytogenes* CTC1034 and *L. sakei* CTC494 grown in monoculture conditions were estimated by fitting the Logistic growth models (Eq. 1, (Rosso et al., 1996)) without ($\lambda = 0$) and with delay ($\lambda > 0$) to the decimal logarithmic transformation of the respective observed counts. The need of the lag time (λ) was assessed with the F-test (Dalgaard, 1995). The *nls* and *nls2* functions from the respective *nls* and *nls2* R packages (R Core Team, 2019) were used in order to obtain the estimates of the primary kinetic parameters, their standard errors and the goodness of fit indicators (see section 2.8).

$$If\ t < \lambda, \qquad Log(N_t) = Log(N_0) \qquad \text{Eq. (1)}$$

$$If\ t > \lambda, \qquad Log(N_t) = Log\left(\frac{N_{max}}{1 + \left(\frac{N_{max}}{N_o} - 1\right) \cdot \left(exp\left(-\mu_{max} \cdot (t - \lambda)\right)\right)}\right)$$

where t is time (days); N_0 is the bacterial concentration (cfu/g) at time zero; N_t is the bacterial concentration (cfu/g) at time t, N_{max} is the maximum population density (cfu/g), λ is the lag time (d) and μ_{max} is the maximum specific growth rate (d^{-1}).

2.5. Modelling the effect of L. sakei *CTC494 on* L. monocytogenes *CTC1034 growth in coculture conditions by microbial interaction models*

To study the interaction phenomenon between *L. monocytogenes* due to the growth of *L. sakei* CTC494, different interaction models were fitted to observed data in coculture experiments. With this approach, values of the interaction parameters could be properly estimated as

suggested by Cornu et al. (2011), and then used as mathematical indicators of the nature of the microbial interaction.

The simultaneous growth of *L. monocytogenes* CTC1034 and *L. sakei* CTC494 during the storage of cooked ham at 2, 5, 10 and 15 ºC was analysed through the fitting of the 4 microbial interaction models, i.e. the simple Jameson-effect model (Eq.2), two modified Jameson-effect models (with interaction γ factor and with N_{cri}, Eqs. 3 and 4, respectively) and the Lotka-Volterra model (Eq. 5) as shown in Table 1. The Jameson-effect model was originally used to predict how the growth of all microbial populations (including pathogens) stops when the dominant microbial population (i.e. lactic acid bacteria, in this work *L. sakei* CTC494) reach its maximum population density *(N_{max}).* Under this Jameson effect, the other growth kinetic parameters of the pathogen, such as lag time and growth rate, remain unchanged by the dominant microbial population and thus can be determined from monoculture experiments (Cornu et al., 2011; Jameson, 1962). In the present work, interaction models based on the Jameson effect were used to fit the data and estimate the corresponding kinetic parameters under coculture conditions. In additions, the use of modifications in the Jameson-effect model were proposed to allow to estimate the behaviour of the pathogen (growth/no growth) after the lactic acid bacteria strain reaches the stationary phase. In this respect, the use of the interaction parameter γ allows the quantification of the inhibiting effect of the bioprotective lactic acid bacteria strain on *L. monocytogenes* growth as a function of temperature (Cadavez et al., 2019; Cornu et al., 2011; Giménez & Dalgaard, 2004; Møller et al., 2013). The estimation of the maximum critical concentration parameter (N_{cri}) refers to the level that *L. sakei* CTC494 should achieve to inhibit the growth of *L. monocytogenes* (Jameson, 1962; Le Marc et al., 2009; Vasilopoulos et al., 2010). Finally, the use of the simple Lotka-Volterra model (Cornu et al., 2011; Fujikawa et al., 2014; Giuffrida et al., 2008) allowed to estimate how the N_{max} of the bioprotective *L. sakei* CTC494 affected the growth of the *L. monocytogenes* CTC1034 through the competition factor. Depending on the value of the competition factor parameters of *L. sakei* CTC494 on *L. monocytogenes* CTC1034 of the Lotka-Volterra model (F_{LsLm} and F_{LmLs}), *L.*

monocytogenes could stop growing (F_{LsLm} =1), grow with reduced μ_{max} (0 < F_{LsLm} < 1) or decline population when *L. sakei* reached its N_{max} (F_{LsLm} >1).

Ordinary differential equations (ODE) included in the interaction models were solved analytically using the numerical Runge-Kutta method (Butcher, 2003) and minimizing the residual sum of squares (RSS) of the errors throughout the search of the most suitable parameter of the interaction model. Parameter estimation by least-square optimization was performed with the "*deSolve*" and "*FME*" packages implemented in the R software (R Core Team, 2019; Cornu et al., 2011).

2.6 Secondary modelling

Secondary modelling was applied to assess the effect of the storage temperature on the primary growth parameters of *L. monocytogenes* CTC1034 and *L. sakei* CTC494 in cooked ham obtained through the fitting of the Logistic growth models to monoculture data and with the fitting of the interaction models to coculture data. For coculture conditions, the impact of the *L. sakei* CTC494 inoculum level on the primary growth parameters of both microorganisms was also assessed.

The hyperbola model (Zwietering et al., 1994) was used to fit the lag time (λ) values determined at different temperatures.

$$\lambda = {a_1}/{(T - b_1)} \qquad \text{Eq. (6)}$$

where a_1 and b_1 are constant parameters and T is the storage temperature (ºC)

The square root model (Eq. 7) (Ratkowsky et al., 1982) was used to estimate the effect of the storage temperature on the growth rate (μ_{max}) of *L. monocytogenes* CTC1034 and *L. sakei* CTC494 obtained in the primary modelling.

$$\sqrt{\mu_{max}} = a_2 \cdot (T - b_2) \qquad \text{Eq. (7)}$$

where a_2 is a constant parameter, T (ºC) is the storage temperature and b_2 is T_{min} (ºC) corresponding to the theoretical minimum growth temperature for each microorganism.

The effect of storage temperature on the maximum population density of the microorganism (N_{max}) was described by using a second degree polynomial equation as in Eq. 8

$$Log\ N_{max} = a_3 \cdot T^2 + b_3 \cdot T + c \qquad \text{Eq. (8)}$$

where a_3 and b_3 are slope parameters and c corresponds to N_{max} at 0 ºC.

The fit of the secondary models was conducted with *nls* and *nls2* functions from the respective *nls* and *nls2* packages included in the R software (R Core Team, 2019).

2.7 Goodness of fit and predictive model performance

Parameter estimates from the models were evaluated with the standard error. Moreover, for the interaction models, the significance of the parameter (*p*-value) was recorded. For all models, the goodness of fit was assessed in terms of RMSE. The adjusted coefficient of determination (R^2_{adj}) was also used to assess the goodness of fit of secondary the linear models (i.e. Eq 7 and Eq8).

To assess the predictive performance of the interaction models for *L. sakei* CTC494 and *L. monocytogenes* CTC1034, an independent experiment was carried out. The two microorganisms were inoculated in the same type of cooked ham and ratios (10:10, 10:10^3 and 10:10^5 cfu/g), vacuum packaged as described above and exposed to 2 non-isothermal profiles, one with mean temperature 2.99 ºC and range from 2.4 ºC to 9.1 ºC (profile 1) and another with mean temperature 3.62 ºC ranging between 0 ºC and 20 ºC (profile 2). The growth of the bioprotective culture and the pathogen was monitored as described in section 2.3. To simulate simultaneous growth of both microorganisms in the two non-isothermal profiles, interaction growth models were applied using appropriate secondary models and specific values of the kinetic parameters (N_{max}) obtained in monoculture and interaction parameter (γ) values derived from the experiments under coculture conditions at constant temperatures. The predictive performance was evaluated with the acceptable simulation zone (ASZ) approach. Model simulation was considered acceptable when at least 70% of the predictions were within the ASZ. In this case, the intrinsic variability of the *L. monocytogenes* data when challenged with *L. sakei* CTC494 (in some cases higher than 1 Log) implied to define the ASZ as the difference of ± 1 Log unit between the observed and predicted bacterial concentration by the developed model as suggested by Møller et al.(2016).

3. Results and discussion

3.1 Growth of L. sakei *CTC494 and* L. monocytogenes *CTC1034 in monoculture conditions*

3.1.1 Primary modelling

Growth of *L. sakei* CTC494 and *L. monocytogenes* CTC1034 in monoculture are shown in Figure 1 (a-d and e-h, respectively) with the estimated kinetic parameters obtained from the fitting of the Logistic growth models without and with delay to Log count data reported in Table 2. No significant lag time (λ) was observed for the bioprotective *L. sakei* CTC494, indicating that this strain was well adapted. On the contrary, *L. monocytogenes* CTC1034 required a time for adaptation before starting to grow and the λ increased with decreasing the storage temperature, being statistically significant for all conditions assayed. Moreover, λ of *L. monocytogenes* was influenced by the *L. sakei* CTC494 initial concentrations, as also reported by Quinto et al. (2016) and Mejlholm & Dalgaard (2015).

For both microorganisms, higher growth rates (μ_{max}) were obtained with increasing the storage temperature. At higher temperatures (10 and 15 ºC), higher μ_{max} were found for *L. monocytogenes* CTC1034 compared to those observed for *L. sakei* CTC494, indicating that *L. monocytogenes* presented a better ability to grow in cooked ham stored at abusive storage temperatures when grown in monoculture. The opposite happened at the lowest temperature tested, where higher μ_{max} was found for *L. sakei* CTC494 compared to the pathogen. Besides the effect on the μ_{max}, the storage temperature did not significantly affect the maximum population density (N_{max}) of the studied bacteria, with an average close to 7 Log cfu/g for *L. sakei* CTC494 and 8 Log cfu/g for *L. monocytogenes* CTC1034. Therefore, when grown in monoculture without interaction, the pathogen generally grew faster and achieved a higher population density than the bioprotective culture, except at 2ºC in which *L. monocytogenes* grew slower that the *L. sakei* CTC494.

3.1.2 Secondary modelling

As *L. sakei* CTC494 started immediately to grow in all conditions, the effect of the storage temperature on the λ was only studied for the *L. monocytogenes* strain. The increase of λ with decreasing the storage temperatures was non-linear and it could be properly quantified throughout the fit of the hyperbola model to the estimated λ (Eq. 6, Table 4).

The square root model (Eq. 7, Table 4) was used to quantify the effect of the storage temperature on the μ_{max}. Though *L. sakei* CTC494 grew faster than *L. monocytogenes* at lower temperatures, the bioprotective LAB was less sensitive to temperature changes as shown in Figure 2, i.e. the slope of the secondary model was steeper for the pathogen and thus at the higher temperatures assessed the growth rate of the pathogen was above that of *L. sakei* CTC494. It is worth to highlight that the growth rates observed for the bioprotective strain *L. sakei* CTC494 were notably lower than the growth predicted for spoilage *Lactobacillus* (not specifically producing bacteriocins) by the model "Growth of *Lactobacillus* in seafood and meat products" included in the Food Spoilage and Safety Predictor (FSSP v4.0) (Mejlholm & Dalgaard, 2007; Mejlholm et al., 2015; Mejlholm & Dalgaard, 2015) considering the same physicochemical characteristics as those of the cooked ham of the present study (pH of 6.07, 2.71% water phase salt and 7034 ppm endogenous lactic acid) (Figure 2). On the contrary, the meat *L. monocytogenes* CTC1034 strain used in the present work showed higher growth capability than the predicted by the "Growth of *Listeria monocytogenes* in chilled seafood and meat products" model of the FSSP. This finding is also in agreement with the previous work (Serra-Castelló et al., 2018) where the *L. monocytogenes* CTC1034 was also shown to grow faster in vacuum packaged cooked ham than the strain 12MOB089LM recommended by the EU Reference laboratory for conducting challenge test studies in refrigerated meat products (EURL Lm, 2014). Thus, considering all these results, the present work would cover a worse-case scenario of a fast-growing *L. monocytogenes*.

Regarding N_{max}, the lack of fit obtained when fitting the polynomial model (Eq. 8) to N_{max} parameters (data not shown) corroborated that storage temperature did not significantly affect

the N_{max} parameter of either *L. sakei* CTC494 and *L. monocytogenes* CTC1034. Therefore, the mean of the estimated N_{max} obtained for all temperatures was assumed to be representative.

3.2 Behaviour of L. sakei *CTC494 and* L. monocytogenes *CTC1034 in coculture conditions*

Growth of *L. monocytogenes* CTC1034 in the presence of different initial concentrations of the bioprotective *L. sakei* CTC494 and stored at the different temperatures is shown in Figure 1 (i-t).

Results of the present study showed that *L. monocytogenes* was able to growth in most of the conditions although to a much lower extent than that observed in monoculture conditions and with a higher variability within the observed counts (slightly wider dispersion of data within a growth curve). Microbiological analysis of the cooked ham revealed that endogenous LAB was below 10 cfu/g indicating that the behaviour of *L. monocytogenes* in coculture conditions was mainly conditioned by *L. sakei* CTC494 and not by endogenous bacteria. The main impact of the *L. sakei* CTC494 strain was the decrease of the maximum population density of the pathogen, which is a very relevant parameter determining the risk of listeriosis according to the quantitative microbial risk assessments (QMRA) developed so far (Pérez-Rodríguez et al., 2017).

At higher initial *L. monocytogenes*:*L. sakei* ratio and at lower storage temperatures, the growth of *L. monocytogenes* was more inhibited than at lower ratios and at higher temperatures pointing out that the initial concentration of the dominant microorganism (the bioprotective *L. sakei* strain) and temperature are key factors determining the level of inhibition of the pathogen. This has also been described for the interaction between the pathogen and spoilage LAB (Mellefont et al., 2008). It is worth to highlight the behaviour of *L. monocytogenes* at 2 ºC in the presence of *L. sakei* CTC494. At the ratio 10:10 (i.e. equal low concentration for both the pathogen and the bioprotective culture) the lag phase of *L. monocytogenes* was almost tripled in comparison with the monoculture growth (i.e. from 21.8 days to 56 days), and no growth of *L. monocytogenes* was recorded during the first 8 weeks of storage. The impact of the

bioprotective culture when the initial *L. sakei* CTC494 concentrations were higher than the pathogen (ratios $10:10^3$ and $10:10^5$) was much greater, resulting in the total lack of growth of *L. monocytogenes,* with a slight tendency to die-off during the chill storage. These results suggested that the lower temperature together with the higher initial *L. sakei* concentration exerted additive effects on the *L. monocytogenes* growth inhibition, compromising its viability in the most unfavourable growth conditions. The antilisteria effect can be mainly attributed to the production of specific metabolites with listeria-inhibitory effect (i.e. sakacin K, De Vuyst & Leroy, 2007; Hugas et al., 1995; Leroy et al., 2005; Ravyts et al., 2008), though the eventual exhaustion of critical nutrients may also play a role. Since *L. sakei* CTC494 shows to be a low acidifying strain (Hugas et al., 1995), no relevant changes in the pH of cooked ham were recorded during the storage time (data not shown), thus the influence of other metabolites such as organic acids on the *L. monocytogenes* growth was considered to be much less relevant than sakacin K.

Overall, the above results showed that the bioprotective strain *L. sakei* CTC494 can be used as a food biopreservation strategy for the control of *L. monocytogenes* growth extending thus, the safe shelf-life of cooked ham with minimal impact on the sensory characteristics, as no slime nor gas or off-odours were detected (data not shown), in agreement with (Aymerich et al., 2002). The bioprotective potential of the *L. strain* CTC494 has also been shown for other meat products such as fermented sausages (acting as a starter culture (Hugas et al., 1995; Leroy & De Vuyst, 2003), showing a significant listericidal effect as it coupled with the acid production and the decrease of a_w due to sausage drying) and for fish (Aymerich et al., 2019; Costa et al., 2019).

3.3 Modelling the bioprotective effect of L. sakei *CTC494 on* L. monocytogenes *CTC1034 growth by microbial interaction models*

For storage temperatures above 5 ºC, results of the coculture conditions showed that the inhibition of the *L. monocytogenes* growth occurred when the dominant population, i.e. *L. sakei* CTC494 reached their N_{max} (Figure 1k-t). These results would indicate that the Jameson-effect model could properly fit the simultaneous growth of the bioprotective LAB culture and the

pathogen data. However, at the lowest storage temperature studied (2 ºC) the *L. monocytogenes* behaviour was strongly conditioned by the level of the bioprotective culture (Figure 1i, 1m and 1q). For the ratio 10:10, the pathogen continued growing after *L. sakei* CTC494 reached its N_{max}; while at higher ratios (ratios $10:10^3$ and $10:10^5$), *L. monocytogenes* was unable to grow, showing even a slight die off trend during the storage period. These results suggested the need of models (Table 1, Eq. 3, Eq. 4 and Eq. 5) that shed light on the *L. monocytogenes* behaviour after *L. sakei* CTC494 achieved its N_{max}. Estimated parameters from the different models and their goodness of fit for simultaneous growth of *L. sakei* CTC494 and *L. monocytogenes* CTC1034 in cooked ham stored at 2, 5, 10 and 15 ºC are reported in the Supplementary Table 1. The 3 variants of the Jameson-effect model appeared statistically adequate to quantitatively describe the simultaneous growth of *L. sakei* and *L. monocytogenes* in cooked ham at all studied storage temperatures, since most of the estimated parameters were significant (p-value <0.05). On the contrary, most of the estimated parameters of the Lotka-Volterra model were not significant (p-value >0.05), especially those defining the interaction between both microorganisms (F_{LsLm}, F_{LmLs}). Therefore, according to these data, Lotka-Volterra model was not the most appropriate interaction model to fit the simultaneous growth of *L. sakei* and *L. monocytogenes* in cooked ham.

Regarding the Jameson-effect models, the common estimated parameters from the 3 interaction models (N_0, μ_{max} and N_{max}) were very close and in most of the conditions, models yielded a good fit (low RMSE). Although that, statistically significant differences in the goodness of fit were found when fitting models to *L. sakei* and *L. monocytogenes* data at 2 ºC, being the modified Jameson-effect models with interaction γ factor and with N_{cri} the models that gave the best fit. Both models reported valuable information about the effect of *L. sakei* CTC494 on *L. monocytogenes* growth. The fit of the Jameson-effect model with N_{cri} indicated that while at higher temperatures (10 and 15 ºC) the critical *L. sakei* CTC494 level at which *L. monocytogenes* stopped growing tended to be lower than the N_{max} of *L. sakei* CTC494, the opposite was predicted at 5 ºC. Thus, these results suggested that at higher temperatures, the bioprotective effect of *L. sakei* CTC494 was the main cause of the decrease of the N_{max} values of

L. monocytogenes. On the other hand, the Jameson-effect model with interaction γ factor, for temperatures ≥ 5ºC, γ parameter was equal to 1 as *L. monocytogenes* stopped growing when *L. sakei* achieved its N_{max}, leading to a simple Jameson-effect model. At 2 ºC, the fit of the model resulted in γ values of 0.19, 1.07 and 1.17 (Table 3) for pathogen:bioprotective strain ratios of 10:10, $10:10^3$ and $10:10^5$, respectively, allowing to properly quantify the growth (γ <1) and the inactivation (γ >1) of *L. monocytogenes* after *L. sakei* CTC494 achieved its N_{max}. Overall, these results agree with those of Costa et al. (2019) dealing with raw fish, which suggest that the interaction between the bioprotective strain and the pathogen could be explained by a combination of two main mechanisms. On the one hand, by the non-specific interaction as considered by the classical Jameson effect; in the present study this was evident for the experiments with initial concentration of *L. sakei* CTC494 higher than that of the pathogen enabling the bioprotective strain to reach the maximum population density before *L. monocytogenes* (Cornu et al., 2011; Mellefont et al., 2008; Jameson, 1962). On the other hand, pathogen inhibition was further enhanced by the specific interaction probably caused by the antagonistic effect of the bacteriocin produced (i.e. sakacin K) by the bioprotective strain (Aguilar and Klotz, 2010; Costa et al., 2019; Vescovo et al., 2006). Moreover, the results showed that this specific interaction was affected by the storage temperature, especially at lower temperature values (< 5 ºC), where *L. monocytogenes* maximum population was not only affected by the initial *L. sakei* concentration but also by the storage temperature. Therefore, the modified Jameson-effect model with interaction γ factor that allows to quantify the inhibiting effect of *L. sakei* CTC494 on the *L. monocytogenes* CTC1034 growth as a function of temperature was chosen for properly characterize the behaviour (either growth and/or slight inactivation) of the pathogen observed at low temperature (2 ºC).

Regarding parameter estimation, results from the fitting of the modified Jameson-effect model with interaction γ factor showed that the growth rates found for *L. sakei* CTC494 and *L. monocytogenes* CTC1034 in coculture conditions (Table 3) were not significantly ($p>0.05$) different from those found in monoculture conditions (Table 2) at the same storage temperature. Therefore, the growth rate of *L. monocytogenes* was not affected by the initial concentrations of

L. sakei CTC494 studied in the present study. These results were in agreement with those reported by the model included in the FSSP application ("Growth of *L. monocytogenes* and *LAB* in chilled seafood and meat products" model) (Mejlholm & Dalgaard, 2007; Mejlholm & Dalgaard, 2015; Mejlholm et al., 2015) where the growth rate of *L. monocytogenes* strains was not affected by the concentration of the *Lactobacillus* strains. On the contrary, the *L. monocytogenes* of N_{max} was significantly reduced with increasing the ratio of *L. sakei*. N_{max} estimates of *L. monocytogenes* were reduced by 2 Log with increasing 100-fold the *L. sakei* initial concentration, emphasizing the greatest effect of *L. sakei* on the N_{max} of *L. monocytogenes*. Therefore, the results indicated that *L. sakei* influenced the growth pattern of *L. monocytogenes* mainly by reducing the maximum population density of the pathogen. Thus, *L. sakei* CTC494 emerged as a feasible bioprotective strategy to control and reduce the *L. monocytogenes* concentration during the cooked ham safe shelf-life in conditions where *L. monocytogenes* could grow.

3.3.1 Quantifying the effect of the storage temperature and L. sakei *CTC494 initial concentration on the primary kinetic parameters of* L. monocytogenes

To further characterize and quantify the impact of the storage temperature and *L. sakei* CTC494 initial concentration on the kinetic parameters obtained from the interaction models, the secondary modelling approach was applied. Same secondary models used in monoculture conditions were used to quantify the impact of storage temperature and initial *L. sakei* CTC494 levels on the kinetic parameters obtained from Jameson-effect model with interaction γ factor (N_0, μ_{max}, N_{max} and γ) describing the simultaneous growth of the bioprotective strain and the pathogen.

Challenging microorganisms in coculture conditions did not significantly affect the growth of *L. sakei* CTC494 compared to monoculture conditions, but affected the growth capability of *L. monocytogenes* CTC1034, mainly by reducing its maximum population density (N_{max}), especially with increasing the initial concentration of the bioprotective strain and lowering the storage temperature (Table 3). In accordance with the observed results, the fit of the squared

root model to the μ_{max} values for *L. sakei* CTC494 and *L. monocytogenes* CTC1034 obtained in coculture conditions was not statistically different from that obtained with the fitting of the model to the observed μ_{max} in monoculture conditions (Table 4). Thus, the growth rate of *L. monocytogenes* was not significantly affected by the presence of *L. sakei* CTC494. On the other hand, while estimates of the maximum population density (N_{max}) for *L. sakei* CTC494 were not affected by either the temperature or the presence of the pathogen with a mean value of 7.72 ± 0.44 Log cfu/g, for *L. monocytogenes* a significant effect of the temperature was observed. In particular, a second degree polynomial model for each ratio was needed to properly describe the effect of temperature on the N_{max} of *L. monocytogenes* (Table 4).

Regarding the Jameson-effect model with interaction γ factor, for temperatures > 5 ºC the γ parameter was not significantly affected by the storage temperature, which is in agreement with the findings of Mejlholm & Dalgaard (2015) on the simultaneous growth of lactobacilli and *L. monocytogenes* in brined shrimp or mayonnaise-based shrimp salad at 5 – 12 ºC. For modelling purposes, γ parameter was equal to 1 for temperatures >5ºC. On the other hand, at 2ºC the estimated γ values for ratios 10:10, $10:10^3$ and $10:10^5$ were <1, very close to 1 and higher than 1, respectively (Table 3). These results suggested that at low temperatures (< 5 ºC) γ could be dependent on temperature.

3.4 Simulation and evaluation of the developed interaction model under non-isothermal conditions.

To evaluate the performance of the developed interaction model, independent data about the simultaneous growth of *L. sakei* CTC494 and *L. monocytogenes* CTC1034 in cooked ham at 3 different pathogen-bioprotective ratios (10:10, $10:10^3$ and $10:10^5$) obtained during the storage of cooked ham at 2 non-isothermal profiles (profile 1: 2.4 - 9.1 ºC and profile 2: 0 - 20 ºC) were used (Figure 3). The temperatures of both dynamic profiles were below 5 ºC during most of the storage time. Within this low temperature range (i.e. 0 to 5 ºC), for the ratio 10:10, *L. monocytogenes* growth was observed after *L. sakei* CTC494 reached its N_{max} in the dynamic profiles 1 and 2, confirming the temperature dependence of γ at low temperature.

The Jameson-effect model with interaction γ factor was used to carry out simulations. Kinetic parameters (secondary models and point-estimate values) and interaction γ factors estimated previously were tested. Due to the temperature dependence of γ at low temperatures, mentioned above, a linear effect of temperature on the γ values from 5 ºC to 2 ºC was assumed for model simulation. In the case of N_{max} , the use of the average from all temperatures under monoculture conditions (8.02 log CFU/g) showed better results, and therefore, this value was used instead of the secondary model including the influence of temperature (Eq 8). Regarding the growth rate, the secondary model obtained for coculture data provided the best results. The simulation with the model showed a good performance (Figure 3) in both non-isothermal profiles, with ≥ 81% of the predicted values being within the ASZ (± 1 Log) (Table 5). Overall, results validated the predictive capacity of the developed mathematical model describing the antilisteria bioprotective effect of *L. sakei* CTC494 during the cooked ham shelf-life, even when exposed to abusive temperatures of storage. The developed model allowed to quantitatively characterize the antilisteria effect of *L. sakei* CTC494 on the N_{max} of *L. monocytogenes* throughout the modified Jameson-effect model with interaction γ factor to properly describe the temperature-dependent effect on the γ parameter at chill temperatures (< 5ºC). Moreover, the application of the predictive model constitutes an useful approach that could be used by food manufacturers to control and optimize the use of the *L. sakei* CTC494 strain as a bioprotective culture, e.g. to determine the concentration of *L. sakei* CTC494 to be added in the product according to foreseeable storage temperature to accomplish with the food safety legislation and/or to extend the safe shelf-life of the product.

4. Conclusions

The new quantitative evidences reported in the present work for the antilisteria strain *L. sakei* CTC494 makes this bioprotective culture a feasible food preservation strategy that can be used by food manufacturers to control *L. monocytogenes* growth in vacuum-packed cooked ham stored under isothermal and non-isothermal conditions, including abusive temperatures. In this sense, the new mathematical model developed in this study could be used by food

manufacturers as a useful approach to optimize the conditions of use of the bioprotective culture with the aim to extend the safe shelf-life of the product.

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Figure captions

Figure 1. Observed counts for *L. sakei* CTC494 (triangles) and *L. monocytogenes* CTC1034 (circles) in cooked ham stored at 2, 5, 10 and 15 ºC in monoculture and coculture conditions. Lines represent the fit of the Logistic growth model without and with delay for the *L. sakei* CTC494 and *L. monocytogenes* CTC1034 growth, respectively, in monoculture conditions and the fit of the Jameson-effect with interaction γ model on the growth of *L. sakei* CTC1034 and *L. monocytogenes* CTC1034 in coculture conditions.

Figure 2. Fit of the square root model (lines) to the growth rates (μ_{max}) found for *L. sakei* CTC494 (triangles, top plot) and *L. monocytogenes* (circles, bottom plot) in monoculture conditions. Closed symbols and continuous lines represent the observed μ_{max} values and the corresponding model fit. Open symbols and dashed lines represent the predictions for lactic acid bacteria and *L. monocytogenes* provided by the FSSP model .

Figure 3. Observed datapoints of *L. sakei* CTC494 (triangles) and *L. monocytogenes* CTC1034 (circles) in cooked ham with 3 different initial concentrations of *L. sakei*, leading to *L. monocytogenes*:*L. sakei* ratios of 10:10, $10:10^3$ and $10:10^5$ cfu/g and stored at 2 different dynamic temperature profiles. Dashed and continuous black lines correspond to the predictions of the modified Jameson-effect with interaction γ model developed in the present study. Dashed and dotted grey lines correspond to the acceptable simulation zone (ASZ) for *L. sakei* and *L. monocytogenes*, respectively, used to compare the observations and predictions. Grey continuous lines stand for the storage temperature recorded.

Table 1. Interaction models evaluated in the present study.

Interaction model	Formula	
Simple Jameson-effect	$t < \lambda_{Ls}, \quad \frac{dN_{Ls}}{dt} = 0$ $t \geq \lambda_{Ls}, \quad \frac{dN_{Ls}}{dt} = N_{Ls} \cdot \mu_{maxLs} \cdot \left(1 - \frac{N_{Ls}}{N_{maxLs}}\right) \cdot \left(1 - \frac{N_{Lm}}{N_{maxLm}}\right)$ $t < \lambda_{Lm}, \quad \frac{dN_{Lm}}{dt} = 0$ $t \geq \lambda_{Lm}, \quad \frac{dN_{Lm}}{dt} = N_{Lm} \cdot \mu_{maxLm} \cdot \left(1 - \frac{N_{Lm}}{N_{maxLm}}\right) \cdot \left(1 - \frac{N_{Ls}}{N_{maxLs}}\right)$	Eq. (2)
Modified Jameson-effect with γ	$t < \lambda_{Ls}, \quad \frac{dN_{Ls}}{dt} = 0$ $t \geq \lambda_{Ls}, \quad \frac{dN_{Ls}}{dt} = N_{Ls} \cdot \mu_{maxLs} \cdot \left(1 - \frac{N_{Ls}}{N_{maxLs}}\right) \cdot \left(1 - \frac{N_{Lm}}{N_{maxLm}}\right)$ $t < \lambda_{Lm}, \quad \frac{dN_{Lm}}{dt} = 0$ $t \geq \lambda_{Lm}, \quad \frac{dN_{Lm}}{dt} = N_{Lm} \cdot \mu_{maxLm} \cdot \left(1 - \frac{N_{Lm}}{N_{maxLm}}\right) \cdot \left(1 - \frac{\gamma \cdot N_{Ls}}{N_{maxLs}}\right)$	Eq. (3)
Modified Jameson-effect with N_{cri}	$t < \lambda_{Ls}, \quad \frac{dN_{Ls}}{dt} = 0$	Eq. (4)

	$t \geq \lambda_{Ls}$, $\frac{dN_{Ls}}{dt} = N_{Ls} \cdot \mu_{maxLs} \cdot \left(1 - \frac{N_{Ls}}{N_{maxLs}}\right) \cdot \left(1 - \frac{N_{Lm}}{N_{criLm}}\right)$	
	$t < \lambda_{Lm}$, $\frac{dN_{Lm}}{dt} = 0$	
	$t \geq \lambda_{Lm}$, $\frac{dN_{Lm}}{dt} = N_{Lm} \cdot \mu_{maxLm} \cdot \left(1 - \frac{N_{Lm}}{N_{maxLm}}\right) \cdot \left(1 - \frac{N_{Ls}}{N_{criLs}}\right)$	
Simplified Lotka-Volterra	$t < \lambda_{Ls}$, $\frac{dN_{Ls}}{dt} = 0$	
	$t \geq \lambda_{Ls}$, $\frac{dN_{Ls}}{dt} = N_{Ls} \cdot \mu_{maxLs} \cdot \left(1 - \frac{N_{Ls} + F_{LsLm} \cdot N_{Lm}}{N_{maxLs}}\right)$	
	$t < \lambda_{Lm}$, $\frac{dN_{Lm}}{dt} = 0$	Eq. (5)
	$t \geq \lambda_{Lm}$, $\frac{dN_{Lm}}{dt} = N_{Lm} \cdot \mu_{maxLm} \cdot \left(1 - \frac{N_{Lm} + F_{LmLs} \cdot N_{Ls}}{N_{maxLm}}\right)$	

where for *L. sakei* CTC494 (*Ls*) and *L. monocytogenes* (*Lm*), λ is the lag time (d), N is the bacterial concentration (Log cfu/g) at time t, μ_{max} is the maximum specific growth rate (d^{-1}), N_{max} is the maximum population density (Log cfu/g), γ is a interaction factor that allows *L. monocytogenes* to increase (γ <1) or decrease (γ >1) after *L. sakei* has reached its N_{max}, N_{cri} is the maximum critical concentration that a population should reach to inhibit the growth of the other population, F_{LsLm} and F_{LmLs} are the competition factors of one species on the other.

Table 2. Estimated parameter values resulting from the fit of the Logistic growth model without and with delay (Eq. 1) to the *L. sakei* CTC494 and *L. monocytogenes* CTC1034 counts, respectively, in monoculture conditions in cooked ham during storage at 2, 5, 10 and 15 ºC.

Microorganism	Temperature (ºC)	Kinetic parameters[a]				Goodness of fit[b]	
		Log N_0 (Log cfu/g)	λ (days)	μ_{max} (Ln/d)	Log N_{max} (Log cfu/g)	n	RMSE
L. sakei CTC494	2	1.76 ± 0.09	-	0.32 ± 0.01	7.21 ± 0.09	37	0.269
	5	1.45 ± 0.08	-	0.64 ± 0.02	7.42 ± 0.07	34	0.218
	10	1.44 ± 0.06	-	1.47 ± 0.03	7.19 ± 0.07	35	0.186
	15	1.53 ± 0.08	-	2.01 ± 0.06	7.02 ± 0.07	34	0.223
L. monocytogenes CTC1034	2	1.24 ± 0.08	21.8 ± 2.6	0.16 ± 0.01	7.50 ± 0.21	37	0.319
	5	1.18 ± 0.11	3.7 ± 0.6	0.84 ± 0.03	8.00 ± 0.09	33	0.275
	10	1.22 ± 0.09	0.7 ± 0.2	2.14 ± 0.05	8.19 ± 0.07	34	0.180
	15	1.39 ± 0.12	0.4 ± 0.1	3.41 ± 0.13	8.38 ± 0.06	33	0.234

[a] Parameter estimate ± standard error. Log N_0 is the initial bacterial concentration (Log cfu/g), λ is the lag time (d), μ_{max} is the maximum specific growth rate (d^{-1}), Log N_{max} is the maximum population density (Log cfu/g).

[b] n: number of data points, RMSE: root mean squared error.

Table 3. Estimated parameter values resulting from the fit of the Jameson-effect model with interaction gamma (γ) factor (Eq. 3) model to the *L. sakei* CTC494 and *L. monocytogenes* CTC1034 counts in coculture conditions in cooked ham during storage at 2, 5, 10 and 15 ºC.

Temperature (ºC)	Ratio	Kinetic parameters[a]								Goodness of fit[b]	
		λ_{-Lm} (d)	$N_{0\text{-}Ls}$ (Log cfu/g)	$N_{0\text{-}Lm}$ (Log cfu/g)	$\mu_{max\text{-}Ls}$ (Ln/d)	$\mu_{max\text{-}Lm}$ (Ln/d)	$N_{max\text{-}Ls}$ (Logcfu/g)	$N_{max\text{-}Lm}$ (Log cfu/g)	γ	n	RMSE
2	10:10	56.0 ± 10.0	1.63 ± 0.06	1.09 ± 0.08	0.31 ± 0.01	0.20 ± 0.02	7.34 ± 0.06	4.75 ± 0.08	0.19 ± 0.04	38	1.000
2	$10:10^3$	-	3.53 ± 0.14	-0.57 ± 0.11	0.41 ± 0.06	0.60 ± 2.12	7.51 ± 0.15	0.03 ± 0.21	1.07 ± 0.22	34	1.012
2	$10:10^5$	-	4.77 ± 0.17	-0.45 ± 0.09	0.46 ± 0.09	0.19 ± 0.49	7.86 ± 0.12	0.33 ± 0.24	1.17 ± 0.43	34	0.876
5	10:10	2.0 ± 0.7 [c]	1.62 ± 0.10	1.09 ± 0.10	0.74 ± 0.02	1.02 ± 0.04	7.51 ± 0.22	7.37 ± 0.60	1.00[d]	42	0.685
5	$10:10^3$	2.8 ± 1.1 [c]	3.72 ± 0.06	-0.96 ± 0.05	0.76 ± 0.04	0.83 ± 0.03	7.86 ± 0.06	4.34 ± 0.06	1.00[d]	40	0.669
5	$10:10^5$	4.2 ± 2.3 [c]	5.21 ± 0.08	0.82 ± 0.11	1.69 ± 0.13	1.48 ± 0.21	7.98 ± 0.08	2.15 ± 0.15	1.00[d]	46	0.692
10	10:10	1.3 ± 0.2 [c]	1.40 ± 0.09	1.32 ± 0.04	1.42 ± 0.05	2.10 ± 0.06	7.01 ± 0.03	8.18 ± 0.05	1.00[d]	40	0.539
10	$10:10^3$	1.2 ± 0.4 [c]	3.78 ± 0.06	-0.20 ± 0.05	1.72 ± 0.13	2.43 ± 0.13	7.55 ± 0.12	5.87 ± 0.11	1.00[d]	40	0.938
10	$10:10^5$	1.4 ± 0.6 [c]	5.03 ± 0.07	-0.18 ± 0.07	2.06 ± 0.11	2.22 ± 0.12	8.09 ± 0.06	3.75 ± 0.12	1.00[d]	40	0.758
15	10:10	0.5 ± 0.1 [c]	1.28 ± 0.18	0.37 ± 0.19	2.39 ± 0.14	3.31 ± 1.44	6.87 ± 0.12	8.22 ± 0.42	1.00[d]	36	0.765
15	$10:10^3$	0.7 ± 0.2 [c]	3.14 ± 0.09	0.66 ± 0.09	2.14 ± 0.13	3.63 ± 0.22	8.21 ± 0.09	6.49 ± 0.07	1.00[d]	38	1.117
15	$10:10^5$	0.0 ± 0.5 [c]	4.99 ± 0.22	0.38 ± 0.14	2.08 ± 0.23	2.66 ± 0.20	8.22 ± 0.26	4.34 ± 0.11	1.00[d]	38	0.976

[a] Parameter estimate ± standard error. Where for *L. sakei* CTC494 (*Ls*) and *L. monocytogenes* (*Lm*), N_0 is the initial bacterial concentration (Log cfu/g), μ_{max} is the maximum specific growth rate (d^{-1}), γ is a interaction factor that allows *L. monocytogenes* to increase (γ <1) or decrease (γ >1) after *L. sakei* has reached its N_{max} and N_{max} is the maximum population density (Log cfu/g).

[b] n: number of data points, RMSE: root mean squared error.

[c] Parameter no significant.

[d] γ parameter was fixed to a value of 1.

Table 4. Fit of the secondary models to the primary kinetic parameters obtained with the Jameson-effect with interaction γ model.

Experimental conditions	Secondary model	Microorganism	Ratio	Parameters[a]			Goodness of fit[b]			
				a_n	b_n	c	n	p	RMSE	R^2_{adj}
Monoculture	$\lambda = {a_1}/{(T - b_1)}$	*L. monocytogenes*	-	11.42 ± 2.22	1.48 ± 0.10	-	4	2	0.604	0.996
	$\sqrt{\mu_{max}} = a_2 \cdot (T - b_2)$	*L. sakei* CTC494	-	0.07 ± 0.05	-6.96 ± 10.62	-	4	2	0.070	0.967
		L. monocytogenes	-	0.12 ± 0.02	-2.56 ± 1.64	-	4	2	0.127	0.960
Coculture	$\sqrt{\mu_{max}} = a_2 \cdot (T - b_2)$	*L. sakei* CTC494	-	0.06 ± 0.03	-9.45 ± 9.70	-	4	2	0.161	0.888
		L. monocytogenes	-	0.09 ± 0.07	-6.09 ± 13.03	-	10	2	0.163	0.958
	$N_{max} = a_3 \cdot T^2 + b_3 \cdot T + c$	*L. monocytogenes*	10:10	-0.04 ± 0.02	0.93 ± 0.30	3.27 ± 1.02	4	3	1.430	0.856
			$10{:}10^3$	-0.06 ± 0.03	1.48 ± 0.52	-2.31 ± 1.74	4	3	2.417	0.870
			$10{:}10^5$	-0.03 ± 0.00	0.74 ± 0.07	-1.00 ± 0.21	4	3	0.297	0.995

[a] Parameter estimate ± standard error. For growth rate the b_2 parameter corresponds to T_{min} (see Eq.7)

[b] n: number of data points, p: number of parameters estimated, RMSE: root mean squared error and R^2_{adj}: adjusted coefficient of determination, R^2_{adj}: adjusted coefficient of determination.

Table 5. Percentage of *L. sakei* CTC494 and *L. monocytogenes* Log counts predicted within the ASZ for the non-isothermal profiles 1 and 2.

Non-isothermal profile	Ratio *Lm*:*Ls*[a]	*L. sakei* CTC494	*L. monocytogenes* CTC1034
1	10:10	100	81
	$10:10^3$	100	93
	$10:10^5$	100	100
2	10:10	100	81
	$10:10^3$	100	83
	$10:10^5$	91	96

[a]*Lm*: *L. monocytogenes*; *Ls*:*L. sakei* CTC494

Figure 1

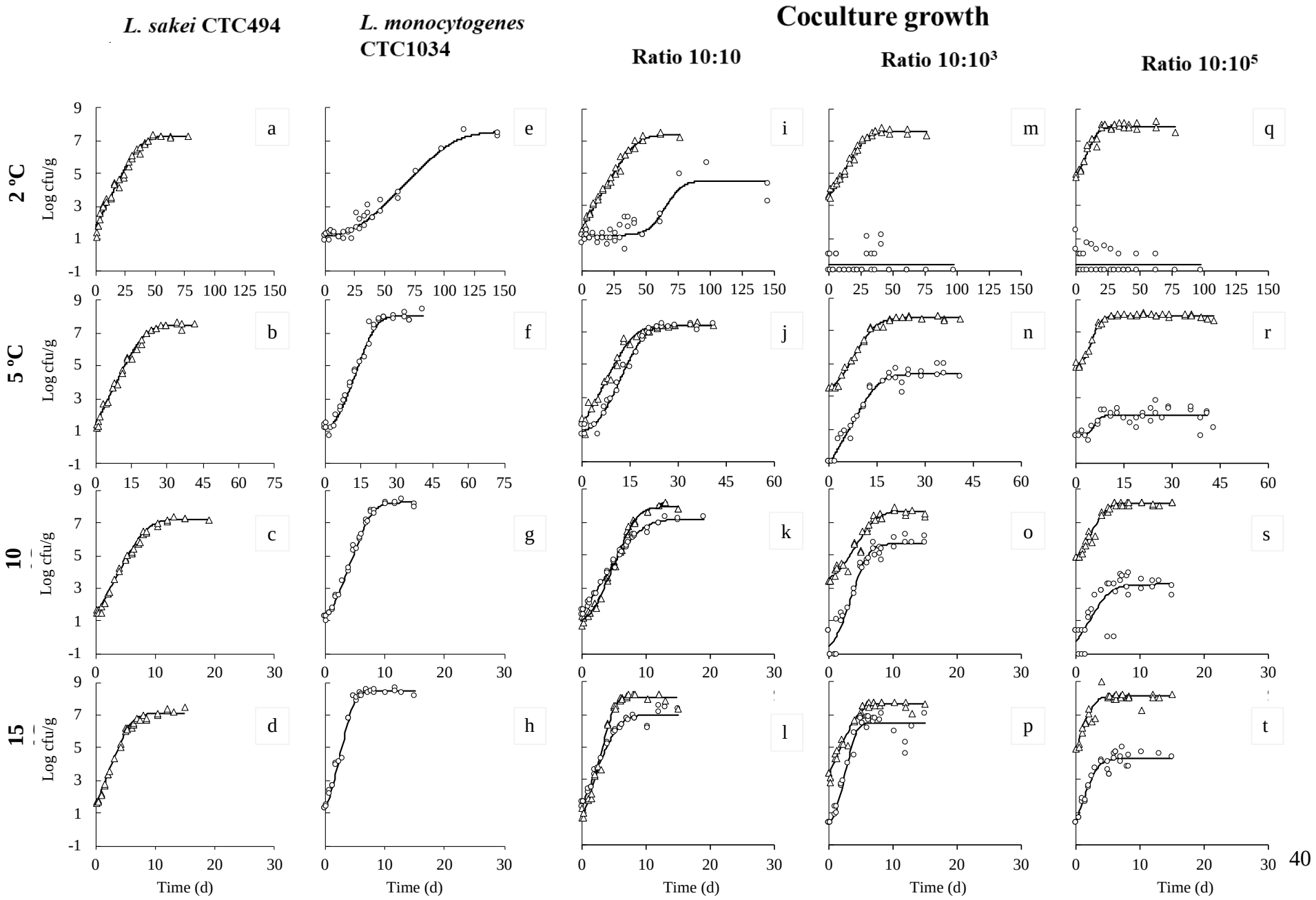

L. sakei CTC494
L. monocytogenes CTC1034
Coculture growth
Ratio 10:10
Ratio 10:10³
Ratio 10:10⁵
2 ºC
5 ºC
10 ºC
15 ºC
Log cfu/g
Time (d)
a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p
q
r
s
t

Figure 2

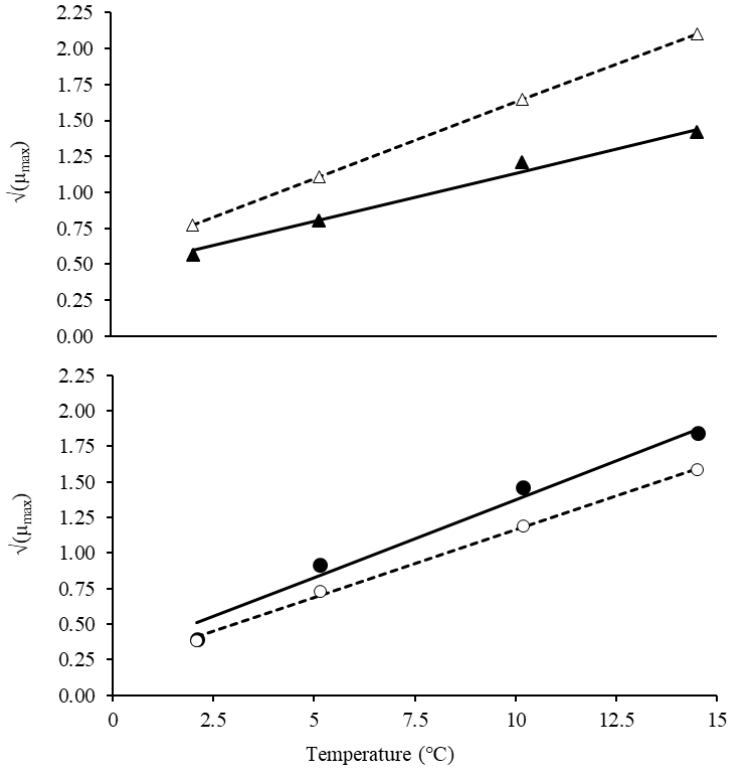
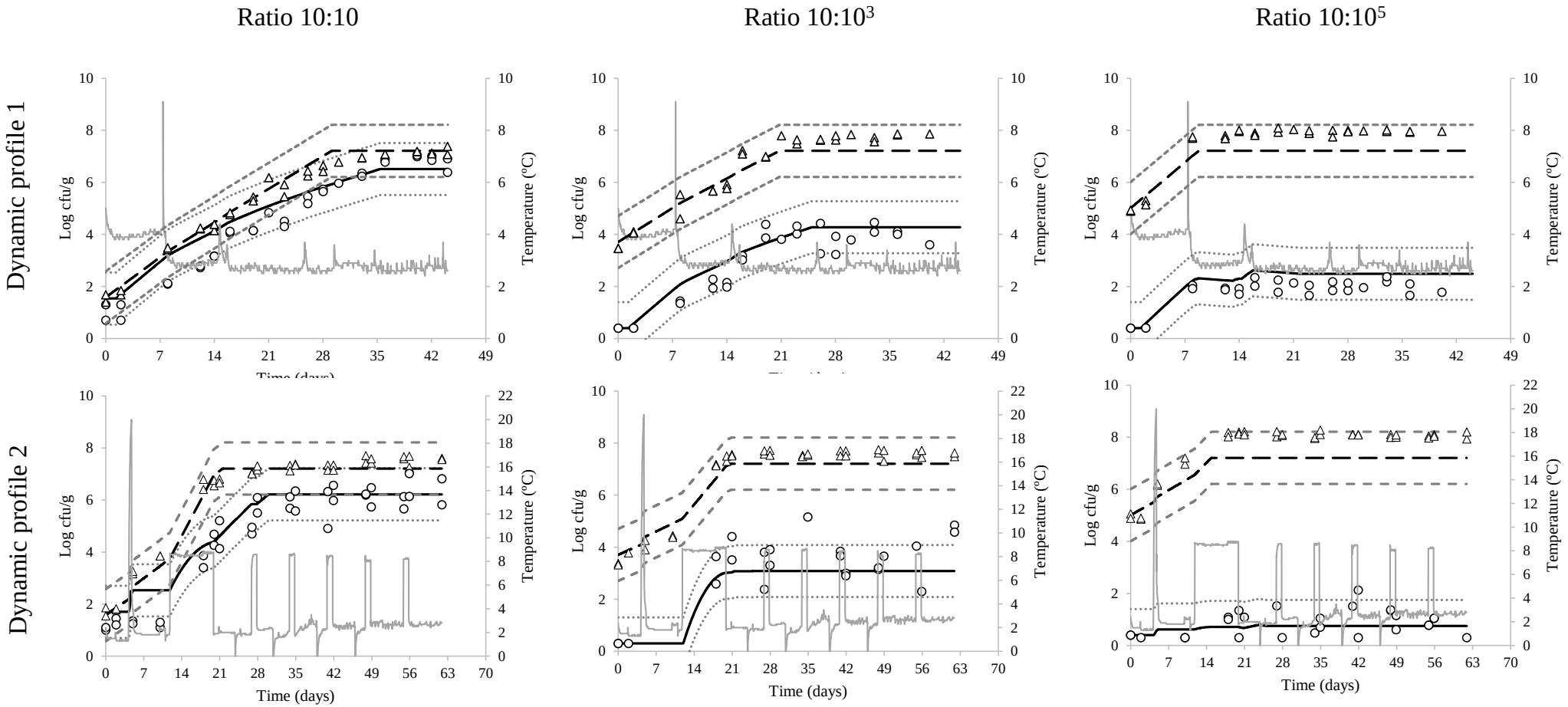

2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
√(μmax)
0
2.5
5
7.5
10
12.5
15
Temperature (°C)

Figure 3


Ratio 10:10
Ratio 10:10^3
Ratio 10:10^5
Dynamic profile 1
Dynamic profile 2
Log cfu/g
Temperature (°C)
Time (days)

Highlights

- *L. sakei* CTC494 is bioprotective against *L. monocytogenes* in vacuum-packaged cooked ham
- *L. sakei* CTC494 has reduced *L. monocytogenes* maximum population density
- *L. sakei* CTC494 totally inhibited pathogen growth at 2 ºC when added at $10{:}10^3$ and $10{:}10^5$ ratio
- An interaction model is provided to establish antilisteria biopreservation strategy