



# A new expanded modelling approach for investigating the bioprotective capacity of *Latilactobacillus sakei* CTC494 against *Listeria monocytogenes* in ready-to-eat fish products

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## ABSTRACT

Understanding the role of food-related factors on the efficacy of protective cultures is essential to attain optimal results for developing biopreservation-based strategies. The aim of this work was to assess and model growth of *Latilactobacillus sakei* CTC494 and *Listeria monocytogenes* CTC1034, and their interaction, in two different ready-to-eat fish products (i.e., surimi-based product and tuna pâté) at 2 and 12 °C. The existing expanded Jameson-effect and a new expanded Jameson-effect model proposed in this study were evaluated to quantitatively describe the effect of microbial interaction. The inhibiting effect of the selected lactic acid bacteria strain on the pathogen growth was product dependent. In surimi product, a reduction of lag time of both strains was observed when growing in coculture at 2 °C, followed by the inhibition of the pathogen when the bioprotective *L. sakei* CTC494 reached the maximum population density, suggesting a mutualism-antagonism continuum phenomenon between populations. In tuna pâté, *L. sakei* CTC494 exerted a strong inhibition of *L. monocytogenes* at 2 °C (<0.5 log increase) and limited the growth at 12 °C (<2 log increase). The goodness-of-fit indexes indicated that the new expanded Jameson-effect model performed better and appropriately described the different competition patterns observed in the tested fish products. The proposed expanded competition model allowed for description of not only antagonistic but also mutualism-based interactions based on their influence on lag time.

## 1. Introduction

The healthy aspect of fish, the accessibility of products and prices together with the “fashion” of different varieties of raw fish or meals based on fish is changing the habits in the European Union (EU) from more traditional meals, especially among the younger consumers (EUMOFA, 2017). Many of these products are designed as ready-to-eat (RTE) and/or lightly preserved food since they are produced from fresh seafood and processed using mild technologies (e.g., sushi and its varieties, marinated or smoked fish, seafood salads or fish pâtés). These food commodities are usually consumed without prior cooking and have been accompanied by various safety concerns due to the risk of microbial contamination during handling and processing (Sheng & Wang, 2021).

RTE foods, and especially those requiring refrigeration, are predominantly associated with the foodborne pathogen *Listeria monocytogenes*, the causative agent of human listeriosis (EFSA Biohaz, 2018). The high public health impact of this pathogen can be highlighted by the severity of listeriosis. In 2019 in EU, 92% listeriosis cases required hospitalization associated with a fatality rate of 17.6% which accounts for more than half of the deaths associated with foodborne diseases (EFSA, 2021). During the last two decades, several studies have documented the occurrence and persistence of this pathogen in RTE fish products and fish processing environments (Elson et al., 2019; Fonnebech Vogel, Huss, Ojeniyi, Ahrens, & Gram, 2001; Thimothe, Nightingale, Gall, Scott, & Wiedmann, 2004; Tocmo et al., 2014). A wide survey, conducted during the period 2010–2011, reported prevalence of *L. monocytogenes* in RTE foods at the EU level was highest in ‘RTE fish’

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(10.3%), followed by 'RTE meat' (2.07%) and 'RTE cheese' (0.47%) at the end of shelf-life (EFSA, 2013). In addition, it has been reported that the growth of *L. monocytogenes* in RTE food at the consumer phase during the shelf-life could account for about one third of the listeriosis cases in the EU (EFSA Biohaz, 2018).

Biopreservation technology is an interesting and cost-effective alternative for shelf-life extension and improvement of food safety (Singh, 2018). It comprises the deliberate use of selected microorganisms and/or their natural metabolites with minimal impact on intrinsic sensory properties of food. Lactic acid bacteria (LAB) are the quintessential microbial group used as protective cultures due to their generally recognized as safe (GRAS) status, as well as their capacity to produce a wide range of antimicrobial compounds (Gao et al., 2019). Besides, many LAB species are included in the qualified presumption of safety (QPS) list (EFSA Panel on Biological Hazards, 2021). In recent years, different studies have focused on developing and testing protective cultures for their application in fish product preservation (Cifuentes Bachmann & Leroy, 2015; Ghanbari, Jami, Domig, & Kneifel, 2013; Gómez-Sala et al., 2016; Leroi et al., 2015; Wiernasz et al., 2017; 2020). In particular, the bioprotective potential of the strain *Lactilactobacillus sakei* CTC494, producer of the bacteriocin sakacin K, against *L. monocytogenes* has been successfully proved in fresh fish (Costa, Bover-Cid, Bolívar, Zurera, & Pérez-Rodríguez, 2019) and smoked fish (Aymerich, Rodríguez, Garriga, & Bover-Cid, 2019; Bolívar et al., 2021) under constant and dynamic refrigeration temperatures. In this regard, factors influencing growth and bacteriocin production are of most importance when using bacteriocinogenic cultures (Gálvez, Abriouel, López, & Omar, 2007). In fact, specific food systems can affect the performance of bacteriocin-producing LAB and/or cause potential loss of bacteriocin activity due to, for instance, physicochemical and food-related factors (pH, temperature, water activity, O<sub>2</sub>, CO<sub>2</sub>, food (micro) structure, buffering capacity...) as well as interaction with food additives or ingredients such as proteins, fat, emulsifiers and nitrites (Gálvez et al., 2007; Said, Ne Gaudreau, Dallaire, Le Tessier, & Fliss, 2019). Hence, understanding the role of the food-related factors on the efficacy of protective cultures is necessary to attain optimal results for developing biopreservation-based strategies.

Studies considering the significance of different food products on microbial interaction between food-borne pathogens and bacteriocinogenic LAB cultures are scarce. Therefore, the aims of this work were i) to evaluate the growth dynamics of *L. sakei* CTC494 and *L. monocytogenes* in mono and coculture conditions in two different fish products (i.e., surimi-based product and tuna pâté) and ii) to quantitatively describe the effect of microbial interaction using microbial competition models.

## 2. Material and methods

### 2.1. Fish products

Two commercial processed RTE fish products (i.e., surimi-based product and tuna pâté) were provided by food producing companies to be used for the challenge tests. The surimi-based product consisted of individual plastic trays containing eight slices packaged under normal (aerobic) atmosphere with an average weight of  $13.5 \pm 0.5$  g per slice. Its ingredient and additives' composition was surimi 49% (with 45% of fish), water, starch, egg white, sunflower oil, salt, sugar, smoke aroma, monosodium glutamate, potassium sorbate and food colouring (carmine and natural candy). The other product corresponded to tuna pâté packaged under normal (aerobic) atmosphere conditions in 25 g-single-dose containers made of semi-rigid aluminium foil. The composition was tuna (35%), sunflower oil, cow milk, potato flakes, water, salt, carrageenan, monosodium glutamate, disodium ribonucleotides, citric acid, acacia gum and aroma. Sliced surimi product and tuna pâté were obtained directly from the producer and kept at refrigeration (2 °C), and room temperature, respectively, up to a maximum of 2 days before the experiments.

### 2.2. Bacterial strains and culture conditions

*L. sakei* strain CTC494 with recognized antilisterial activity was selected as the bioprotective culture in this study (Hugas, Garriga, Aymerich, & Monfort, 1995). The pathogenic species was *L. monocytogenes* strain CTC1034 (serotype 4b) from the culture collection of the Food Safety and Functionality Programme of IRTA, previously used as reference strain to evaluate the microbial inhibition produced by bacteriocins (including sakacin K) (Garriga, Aymerich, Costa, Monfort, & Hugas, 2002). Stock cultures were maintained at -80 °C in cryovials (Microbank™, Pro-LAB Diagnostic, USA) with 20% glycerol (v/v) as cryoprotectant.

A bead of *L. sakei* CTC494 was pre-cultured separately at static conditions for 24 h in de Man, Rogosa, and Sharpe broth (MRS, Oxoid, UK) at 33 °C with 10% CO<sub>2</sub>. For *L. monocytogenes*, a bead of the stock culture was pre-cultured at static conditions for 24 h in Brain Heart Infusion broth (BHI, Oxoid, UK) at 37 °C. Afterwards, a 24 h-subculture was made for each microorganism in the same incubation conditions, followed by a third subculture incubated for 18–20 h (Costa et al., 2019). This process resulted in early stationary phase cultures, with a cell density of ca.  $10^8$  CFU/mL and  $10^9$  CFU/mL for *L. sakei* CTC494 and *L. monocytogenes* CTC1034, respectively.

Before experiments, cultures were twice-washed in phosphate buffered saline solution (PBS) (Medicago AB, Uppsala, Sweden) by centrifugation at 3326 g (Jouan C4i, Thermo Electron Corporation, France) for 10 min and then 10-fold diluted in sterile physiological saline water (PSW, 0.85% w/v NaCl) to obtain the cell density required for the challenge tests (section 2.4) which was confirmed by viable plate counts.

### 2.3. Challenge tests

Four batches were prepared for each fish product: (1) non-inoculated product (control); (2) inoculated with ca.  $10^2$  CFU/g *L. monocytogenes*; (3) inoculated with ca.  $10^2$  CFU/g *L. sakei* CTC494 and (4) inoculated with *L. monocytogenes* and *L. sakei* CTC494 at concentrations of ca.  $10^2$  and  $10^4$  CFU/g, respectively, generating the ratio 1:2 expressed in logarithmic scale. This inoculum ratio was selected based on previous sensory assessments for *L. sakei* CTC494 in fresh fish (Costa et al., 2019) and hot-smoked fish (Bolívar et al., 2021). In both studies, it was demonstrated that the LAB culture did not increase sensory spoilage rates at an initial concentration  $\leq 10^4$  CFU/g when compared to non-bioprotected samples.

Monoculture experiments were carried out to individually assess the growth of each microorganism in both RTE fish products. For that, batches 2 and 3 were used. Slices of surimi-based product were surface inoculated at 1% (v/w) from the appropriate decimal dilution using a L-shaped sterile spreader. Slices were maintained in the safety cabinet for 1 min to allow inoculum absorption. Afterward, surimi slices were individually vacuum packaged (AUDIONVAC, 151HG) using plastic bags (PA/PE 150 µm, 140 × 300 mm, Sacoliva, Barcelona, Spain). Single-dose containers of tuna pâté were inoculated with aliquots of 50 µL (2% v/w) from the appropriate decimal dilution using a sterile syringe with needle (BD Plastipak, Spain) inserted through an adhesive septum (ø 15 mm, PBI Dansensor, Denmark) which was previously placed on the upper central part of the aluminium foil lid.

Coculture experiments were performed to examine the impact of microbial interaction on growth kinetics using batch 4. For surimi samples, *L. sakei* CTC494 was first surface inoculated and maintained in the safety cabinet as described before. *L. monocytogenes* was then spread, followed by a cell attachment period as the LAB strain case. For tuna pâté samples, the inoculation was performed by preparing a previous mixture of *L. sakei* and *L. monocytogenes* at the ratio described before for batch 4 and inoculated as mentioned for monoculture experiments.

Both RTE fish products were stored at two constant temperatures targeted at 2 and 12 °C, representative for fish product distribution and storage (i.e., cold and mild abuse temperature, respectively). Depending

on the microorganism and condition, the storage period ranged from 15 to 50 days at 2 °C and from 6 to 10 days at 12 °C to ensure the stationary growth phase was reached. The storage temperature of all experimental conditions was regularly recorded by data loggers (Fourtec, Mini-LitE5032L, USA), with average values of 2.2 (±0.21) and 12.2 (±0.61) °C. Growth experiments were carried out in duplicate.

#### 2.4. Microbiological analyses

Individual slices of surimi product and single-dose containers of tuna pâté were considered the analytical samples. The growth of *L. sakei* CTC494 and *L. monocytogenes* CTC1034 was enumerated by plate count methods. For that, two samples were aseptically taken at each sampling point, homogenized 1:10 for 60 s (1500 rpm) in a stomacher bag (Masticator, IUL Instruments, Spain) and subsequently diluted 10-fold in PSW (0.85% NaCl). *L. sakei* was enumerated by pour plating in MRS agar (Oxoid) supplemented with bromocresol purple (BP, 0.12 g/L, Sigma-Aldrich, USA) and incubating with overlay at 33 °C under 10% CO<sub>2</sub> for 48 h. BP is a pH indicator used for the enumeration of LAB in foods that indicates the production of lactic acid by changing the MRS colour from purple to yellow (Sobrun, Bhaw-Luximon, Jhurry, & Puchooa, 2012). *L. monocytogenes* was enumerated by surface plating on *Listeria* selective agar base (Oxford formulation; Oxoid) containing selective supplement (SR140E; Oxoid) and incubating at 37 °C for 48 h (method ISO 11290–2).

#### 2.5. Physicochemical analyses

Water activity ( $a_w$ ) of surimi product and tuna pâté was determined in triplicate from non-inoculated samples (batch 1) using an  $a_w$ -meter AquaLab 4 (Decagon Devices Inc. Pullman, WA, USA). Lactic acid concentration and pH were determined in the studied fish products from batch 4 (coculture) on the initial and final experimental day. The pH of the fish products was measured using a portable pH-meter (Hanna Edge, HI2020, USA) with a penetration probe (HI10530). The analysis of lactic acid was performed in duplicate from 5 g fish sample, after the extraction with 0.15 M perchloric acid, by HPLC using an ion exclusion column (Transgenomic IC9SepICE-ORH-801, Chrom Tech. Inc., MN, USA) with a refractive index (RI) detector (Hereu, Dalgaard, Garriga, Aymerich, & Bover-Cid, 2014). An ANOVA ( $p \leq 0.05$ ) was performed in MS-Excel software (Microsoft Corp., Redmond, WA, USA) to determine significant differences on lactic acid concentration between the starting and final day of the storage period.

#### 2.6. Mathematical modelling of microbial interaction

The mathematical approach used, in this study, to investigate the interaction effect between *L. sakei* CTC494 and *L. monocytogenes* in two fish products and describe inter-species competition was based on (i) the determination of the kinetic parameters of the two microbial populations from experimental growth curves obtained in monoculture and coculture; (ii) the simulation of simultaneous growth based on microbial competition models using the monoculture kinetic parameters and (iii) the assessment of the performance of the tested microbial competition models to describe the observed microbial data by suitable statistical indexes.

##### 2.6.1. Primary model fitting

The Baranyi and Roberts (1994) primary growth model was fitted to microbial growth curves obtained in mono and coculture conditions for *L. sakei* CTC494 and *L. monocytogenes* at the studied storage temperatures (2 and 12 °C). This model was used to obtain the kinetic growth parameters lag time ( $\lambda$ , h), maximum specific growth rate ( $\mu_{max}$ , h<sup>-1</sup>) and maximum population density ( $N_{max}$ , log CFU/g). Model parameters were estimated using the lsqcurvefit routine of the Optimisation Toolbox of Matlab version R2020b (The MathWorksInc®, Natick, USA). Standard

errors of parameter estimates were calculated from the Jacobian matrix obtained in the fitting process.

In addition, an F-Test ( $p \leq 0.05$ ) was performed in MS-Excel to prove model adequacy in data fitting for mono and coculture conditions. An F-value inferior to the critical percentile of the F distribution indicates that the model was not statistically accepted to fit the data set (Gil, Miller, Brandão, & Silva, 2017).

To assess the influence of product type on the obtained growth kinetic parameters ( $\lambda$ ,  $\mu_{max}$ ,  $N_{max}$ ), the Student's T-Test ( $p \leq 0.05$ ) was used to compare the two means (independent samples for surimi product vs. tuna pâté) in MS-Excel. Then, the T-Test was also applied to assess differences between growing conditions (monoculture vs. coculture) on the kinetic parameters.

##### 2.6.2. Modelling microbial interaction in RTE fish products

The modelling approach used to describe the simultaneous growth of *L. sakei* CTC494 and *L. monocytogenes* in the fish products was primarily built on the Jameson-effect interaction. This phenomenon determines that two microbial populations in mixed culture simultaneously stop growing when the maximum population density of the total population is achieved (Jameson, 1962), which may be result from non-specific competition between populations for a common limiting resource. To model this interaction, a generic primary growth model coupled with the logistic deceleration function can be used, that is the so-called Jameson-effect model. The logistic deceleration describes an empirical self-limiting growth process that represents the exhaustion of critical resources and the accumulation of growth inhibitory by-products (Cadavez et al., 2019). Giménez and Dalgaard (2004) modified the deceleration function that describes interaction between populations by including the maximum population density of each competing population. Similarly, this approach considers that one population stops growing when the other has reached its maximum population density. It can be written as represented by Eq. (1), standing subscript '1' for the population of *L. sakei* and subscript '2' for the population of *L. monocytogenes*.

$$\begin{cases} \frac{dN_1}{dt} = \mu_{max1} N_1 \left(1 - \frac{N_1}{N_{max1}}\right) \left(1 - \frac{N_2}{N_{max2}}\right) \frac{Q_1}{1 + Q_1} \\ \frac{dQ_1}{dt} = \mu_{max1} Q_{1t-1} \\ \frac{dN_2}{dt} = \mu_{max2} N_2 \left(1 - \frac{N_2}{N_{max2}}\right) \left(1 - \frac{N_1}{N_{max1}}\right) \frac{Q_2}{1 + Q_2} \\ \frac{dQ_2}{dt} = \mu_{max2} Q_{2t-1} \end{cases} \quad (1)$$

where  $N$  is the cell concentration (CFU/g) at time  $t$ ,  $\mu_{max}$  is the maximum specific growth rate (h<sup>-1</sup>),  $N_{max}$  is the maximum population density (CFU/g);  $Q$  quantifies the physiological state of cells at time  $t$  allowing for description of the lag time.

The value of  $Q$  at  $t = 0$  ( $Q_0$ ) was calculated for both microorganisms as follows (Baranyi & Roberts, 1994):

$$Q_0 = \frac{1}{e^{(\mu_{max}\lambda)} - 1} \quad (2)$$

where  $\lambda$  (h) is the lag time duration of the bacterial population.

The Jameson-effect hypothesis is, of course, not applicable to every interaction between two microbial populations. As stated by Cornu, Billoir, Bergis, Beaufort, and Zuliani (2011), "To circumvent cases in which the simplistic hypothesis of simultaneous deceleration is not applicable, variants of the Jameson-effect models have been proposed". The observations in our study demonstrate that microbial populations showed different competition patterns depending on the fish product and storage temperatures. Therefore, the expanded version of the Jameson-effect model as proposed by Møller et al. (2013) was used in this study (Eq. (3)).

**Table 1**Lactic acid concentration and pH values ( $\pm$ standard deviation) obtained in the ready-to-eat (RTE) fish products under study.

| Product       | Batch          | T (°C)           | Storage time | Lactic acid (%)  | pH              |
|---------------|----------------|------------------|--------------|------------------|-----------------|
| Sliced surimi | 4 (Co-culture) | N/A <sup>a</sup> | Initial      | 2.41 $\pm$ 0.01  | 6.76 $\pm$ 0.32 |
|               |                | 2                | Final        | 1.45 $\pm$ 0.04  | 5.89 $\pm$ 0.50 |
|               |                | 12               | Final        | 1.80 $\pm$ 0.10  | 6.10 $\pm$ 0.33 |
| Tuna pâté     | 4 (Co-culture) | N/A              | Initial      | 0.75 $\pm$ 0.01  | 6.04 $\pm$ 0.01 |
|               |                | 2                | Final        | 0.95 $\pm$ 0.11* | 6.13 $\pm$ 0.07 |
|               |                | 12               | Final        | 0.78 $\pm$ 0.02  | 5.76 $\pm$ 0.06 |

<sup>a</sup> Not applicable (N/A).\* This value was significantly higher compared to the initial storage day ( $p \leq 0.05$ ).

$$\begin{cases} \frac{dN_1}{dt} = \mu_{max1} N_1 \left(1 - \frac{N_1}{N_{max1}}\right) \left(1 - \frac{\gamma_{12} \times N_2}{N_{max2}}\right) \frac{Q_1}{1 + Q_1} \\ \frac{dQ_1}{dt} = \mu_{max1} Q_{1t-1} \\ \frac{dN_2}{dt} = \mu_{max2} N_2 \left(1 - \frac{N_2}{N_{max2}}\right) \left(1 - \frac{\gamma_{21} \times N_1}{N_{max1}}\right) \frac{Q_2}{1 + Q_2} \\ \frac{dQ_2}{dt} = \mu_{max2} Q_{2t-1} \end{cases} \quad (3)$$

where  $\gamma_{12}$  and  $\gamma_{21}$  are the interaction coefficients measuring the effect of *L. sakei* CTC494 on *L. monocytogenes* and vice-versa, respectively; other parameters are as indicated for Eq. (1).

This approach is more flexible than the classical Jameson-effect model since it includes an interaction parameter ( $\gamma$ ) that must be estimated from microbial growth curves in coculture. The parameter  $\gamma$  defines the inhibiting effect of the dominant population on the other population which may differ among environmental conditions. In this way, Eq. (3) allows to describe the concentration of *L. monocytogenes* either when (i) it increases after *L. sakei* has reached its  $N_{max}$  ( $\gamma_{12} < 1$ ) or (ii) it decreases after *L. sakei* has reached its  $N_{max}$  ( $\gamma_{12} > 1$ ). With an  $\gamma$ -value = 1.0, Eq. (3) and the classical Jameson-effect model (Giménez & Dalgaard, 2004) are equivalent (i.e., where the concentration of *L. monocytogenes* stabilizes to the maximum population density value after *L. sakei* has reached its  $N_{max}$ ).

The Jameson-effect hypothesis has been traditionally based on microbial interactions in foods that only limit the maximum population density, without any significant effect on the lag time or maximum growth rate (Cauchie et al., 2020). When mutual interaction occurs, microbial lag time may be affected, resulting in earlier entry into the exponential growth phase (Yang et al., 2017). To reflect the effect of microbial interaction on lag time, we propose a modification of the adjustment function  $Q(t)$  by including an empirical parameter ( $\beta$ ) that needs to be fitted from coculture experiments. In this way, if  $\beta_{12} < 0$ , population 1 shortens the lag time of population 2 and vice-versa, describing a mutualism-based interaction between the two populations. The new expanded Jameson-effect model can be expressed as follows:

$$\begin{cases} \frac{dN_1}{dt} = \mu_{max1} N_1 \left(1 - \frac{N_1}{N_{max1}}\right) \left(1 - \frac{\gamma_{12} \times N_2}{N_{max2}}\right) \frac{Q_1}{1 + Q_1} \\ \frac{dQ_1}{dt} = \mu_{max1} Q_{1t-1} \left(1 - \beta_{12} \frac{N_2}{N_1}\right) \\ \frac{dN_2}{dt} = \mu_{max2} N_2 \left(1 - \frac{N_2}{N_{max2}}\right) \left(1 - \frac{\gamma_{21} \times N_1}{N_{max1}}\right) \frac{Q_2}{1 + Q_2} \\ \frac{dQ_2}{dt} = \mu_{max2} Q_{2t-1} \left(1 - \beta_{21} \frac{N_1}{N_2}\right) \end{cases} \quad (4)$$

where  $\beta_{12}$  and  $\beta_{21}$  are the interaction coefficients describing the effect of population 1 on the lag time of population 2 and vice-versa, respectively; other parameters are as indicated for Eq. (1) and (3).

## 2.7. Evaluation and interaction models fitting

The performance of the microbial interaction models to describe the experimental data was assessed by the goodness-of-fit indexes root mean squared error (RMSE) and corrected Akaike Information Criterion (AICc).

$$RMSE = \sqrt{\frac{\sum (\log N_{obs} - \log N_{est})^2}{n - p}} \quad (5)$$

$$AICc = n \cdot \ln \left( \frac{\sum (\log N_{obs} - \log N_{est})^2}{n} \right) + 2 \cdot (p + 1) + \frac{2 \cdot (p + 1) \cdot (p + 2)}{n - p - 2} \quad (6)$$

where  $\log N_{obs}$  and  $\log N_{est}$  is the log-transformed cell concentrations observed and estimated with the model, respectively,  $n$  is the number of observations, and  $p$  is the number of model parameters.

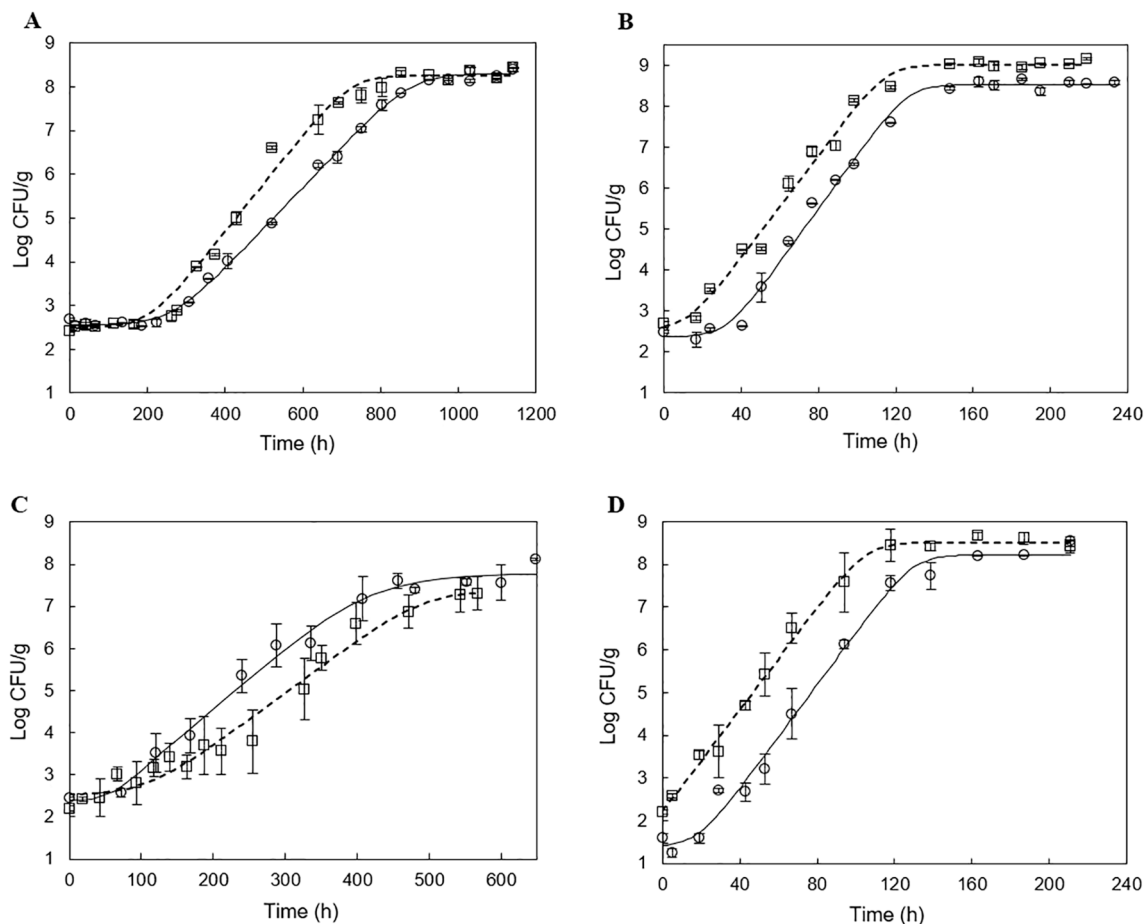
The fitting process of microbial interaction was performed in Matlab using the previous estimated kinetic parameters in monoculture conditions and optimizing the interaction parameters ( $\gamma$  and  $\beta$ ). For that, the Runge-Kutta method was used to numerically solve the differential equations through the *ode45* function in Matlab. This method was combined with the *fminsearch* function to estimate the interaction parameters by least-squares optimization. The initial guess selection for the parameter optimization algorithm was based on an iterative process, by choosing a wide range of values for each parameter, at the outset, and narrowing them according to the improvement in RMSE. Thus, the initial guess of the expanded Jameson model parameters was  $\gamma_{12}$  and  $\gamma_{21} = 0$  for surimi product and  $\gamma_{12} = 2$  and  $\gamma_{21} = 0$  for tuna pâté. Whereas for the new expanded Jameson model fitting, the chosen starting values of  $\gamma_{12}$ ,  $\gamma_{21}$ ,  $\beta_{12}$  and  $\beta_{21}$  corresponded to 2, 1, 0 and 0, respectively, for surimi product, and 2, 0, 0 and 0, respectively, for tuna pâté. The confidence intervals of the interaction parameters were calculated by bootstrap method.

## 3. Results and discussion

### 3.1. Physicochemical characterization

Surimi-based product and tuna pâté exhibited an initial  $a_w$  of 0.988 ( $\pm 0.002$ ) and 0.987 ( $\pm 0.002$ ), respectively. The pH and lactic acid concentration values obtained in batch 4 (coculture) of the studied fish products are presented in Table 1. No relevant changes in pH were detected during growth experiments in both fish products and storage temperatures.

Regarding the concentration of lactic acid, levels obtained in the tested fish products on the initial experimental day were within the lower range values reported for endogenous lactic acid in other processed and RTE meat and seafood products (Mejlholm et al., 2010). The concentration of lactic acid did not significantly increase ( $p > 0.05$ ) at the end of the experimental period compared to the initial day with the exception of tuna pâté at 2 °C (Table 1).



**Fig. 1.** Experimental growth data of *Lactobacillus sakei* CTC494 (○) and *Listeria monocytogenes* CTC1034 (□), in monoculture, on sliced surimi product (A and B) and tuna pâté (C and D). A and C refer to temperatures of 2 °C and B and D refer to temperatures of 12 °C. Solid and dashed lines correspond to the fit of the Baranyi and Roberts (1994) model for *L. sakei* and *L. monocytogenes*, respectively. Error bars represent the standard deviation of two independent experiments with duplicate samples.

**3.2. Microbial growth in monoculture**

Fig. 1 presents the experimental growth data of *L. sakei* CTC494 and *L. monocytogenes* obtained in monoculture at 2 and 12 °C in sliced surimi product (A-B) and tuna pâté (C-D). The initial concentration of both microorganisms in the fish products ( $t = 0$  h) was around 2.5 log CFU/g except for *L. sakei* CTC494 at 12 °C in tuna pâté which was a bit lower ( $1.6 \pm 0.5$  log CFU/g), though quantifiable by plate count methods.

Table 2 summarizes the kinetic parameters estimated from Baranyi and Roberts (1994) model for the four sets of experimental growth curves. The primary growth model adequately described the experimental growth data in monoculture according to the F-test and the

goodness-of-fit indexes, with low RMSE values (<0.32).

Comparing microbial kinetics in the studied fish products, the parameter lag time  $\lambda$  in monoculture was significantly shorter ( $p \leq 0.05$ ) in tuna pâté than in surimi product for both microorganisms and storage temperatures, although this trend was much remarkable at 2 °C. The longer lag time in surimi product may be related to the fact that this product was vacuum packaged as well as the presence of potassium sorbate in the formulation, both known to delay the growth of *L. monocytogenes* compared with normal (air) atmosphere packaging (Pal, Labuza, & Diez-Gonzalez, 2008) and the absence of sorbate (González-Fandos & Domínguez, 2007) in tuna pâté. In addition, other compositional differences between the tested fish products include the

**Table 2**

Kinetic parameters and their standard errors estimated from the model of Baranyi and Roberts (1994) for mono and coculture experiments.

| Product       | Microorganism           | T (°C) | $\lambda$ (hours) |                  | $\mu_{max}$ (h <sup>-1</sup> ) |               | $N_{max}$ (log CFU/g) |             | RMSE <sup>a</sup> |       |
|---------------|-------------------------|--------|-------------------|------------------|--------------------------------|---------------|-----------------------|-------------|-------------------|-------|
|               |                         |        | Mono              | Co               | Mono                           | Co            | Mono                  | Co          | Mono              | Co    |
| Sliced surimi | <i>L. sakei</i> CTC494  | 2      | 259.62 (0.62)     | 171.12 (0.67)    | 0.021 (0.008)                  | 0.050 (0.057) | 8.30 (0.08)           | 8.42 (0.28) | 0.096             | 0.275 |
|               |                         | 12     | 32.08 (0.22)      | 63.93 (0.18)     | 0.151 (0.127)                  | 0.257 (0.458) | 8.52 (0.16)           | 8.41 (0.12) | 0.192             | 0.144 |
|               | <i>L. monocytogenes</i> | 2      | 200.64 (0.96)     | 78.54 (0.89)     | 0.025 (0.019)                  | 0.037 (0.045) | 8.26 (0.14)           | 6.61 (0.25) | 0.191             | 0.244 |
|               |                         | 12     | 13.38 (0.29)      | 31.83 (0.26)     | 0.144 (0.135)                  | 0.167 (0.240) | 9.01 (0.20)           | 6.72 (0.23) | 0.234             | 0.278 |
| Tuna pâté     | <i>L. sakei</i> CTC494  | 2      | 48.03 (0.80)      | 75.14 (0.76)     | 0.033 (0.026)                  | 0.053 (0.060) | 7.69 (0.31)           | 7.69 (0.34) | 0.071             | 0.165 |
|               |                         | 12     | 18.25 (0.34)      | 15.32 (0.12)     | 0.141 (0.180)                  | 0.150 (0.128) | 8.23 (0.38)           | 8.06 (0.39) | 0.311             | 0.234 |
|               | <i>L. monocytogenes</i> | 2      | 108.86 (1.37)     | N/A <sup>b</sup> | 0.029 (0.051)                  | N/A           | 7.36 (0.61)           | N/A         | 0.310             | N/A   |
|               |                         | 12     | 0.55 (0.2)        | N/A              | 0.138 (0.055)                  | N/A           | 8.52 (0.20)           | N/A         | 0.189             | N/A   |

<sup>a</sup> Root Mean Squared Error (RMSE).

<sup>b</sup> Not applicable (N/A).

fat content (i.e., 26% fat in tuna pâté and 0.4% fat in surimi product). In relation to this, a recent study investigated the isolated effect of fat content on *L. monocytogenes* growth dynamics in fish-based emulsions and gelled emulsions systems (ranging from 1 to 20% fat) and observed a lag-reducing effect in systems containing > 1% fat at temperatures of 4–10 °C (Verheyen et al., 2020). These authors suggested that this phenomenon was caused by a protective effect of the fat droplets at chilling temperatures.

Regarding the parameter maximum specific growth rate ( $\mu_{max}$ ), there were no significant differences ( $p > 0.05$ ) comparing the fish products for *L. sakei* CTC494 and *L. monocytogenes*. In addition, the two studied microorganisms exhibited similar  $\mu_{max}$  values in each fish product and storage temperature (Table 2). Interestingly, the obtained  $\mu_{max}$  values for *L. monocytogenes* at 2 °C in surimi product and tuna pâté are in accordance with those reported by Verheyen et al. (2020) at 4 °C in fish-based gelled emulsions systems containing 1% fat ( $0.029 \text{ h}^{-1}$ ) and 20% fat ( $0.030 \text{ h}^{-1}$ ). In that study, it was also observed that the  $\mu_{max}$  of the pathogen was not significantly affected by the different fat contents. The fish matrices under study did not have a significant influence ( $p > 0.05$ ) on the maximum population density  $N_{max}$  of the LAB strain or the pathogen.

### 3.3. Microbial growth in coculture

The obtained growth data in coculture and the estimated parameters resulting from the fitting of the Baranyi and Roberts (1994) model are shown in Fig. 2 and Table 2, respectively. According to results from the F-Test, the Baranyi model was not statistically adequate to fit microbial data obtained for *L. monocytogenes* in tuna pâté at both storage

temperatures as limited increase (<1 to 2 log units at 2 and 12 °C, respectively) occurred due to the antagonistic activity of *L. sakei* CTC494.

A shorter  $\lambda$  ( $p \leq 0.05$ ) was observed in tuna pâté compared to sliced surimi product for both microorganisms, in coculture, and storage temperatures, which could be explained, as mentioned in the case of monoculture experiments, by differences in the packaging atmospheres as well as in the food composition, i.e., sorbate and fat content of the studied fish products, causing the latter a possible protective (lag-reducing) effect of the fat droplets in the product with higher fat content (i.e., tuna pâté). The values of  $\mu_{max}$  and  $N_{max}$  obtained for *L. sakei* CTC494 in coculture were similar ( $p > 0.05$ ) to those observed in monoculture experiments in both fish products and storage temperatures. By contrast, the parameter  $\lambda$  at 2 °C for cocultured *L. sakei* and *L. monocytogenes* obtained in sliced surimi product presented a reduction compared to that observed in monoculture. This fact was also observed by Costa et al. (2019) for the same strains in fish juice at 2 °C, who suggested that the differences were rather produced by the fitting process, that was affected by the relatively  $\lambda$  short duration (i.e., low value), than a hypothetical interaction between microorganisms. However, in our study, in which actual fish products were used, a clear and long  $\lambda$  was observed and estimated with low fitting errors (Table 2). By contrast, no significant differences were obtained for the parameter  $\mu_{max}$  in sliced surimi comparing mono and coculture experiments, while the parameter  $N_{max}$  for *L. monocytogenes* was significantly lower ( $p \leq 0.05$ ) in coculture at both assayed temperatures, with a mean drop of 2 log units. In fact, the inhibiting effect of *L. sakei* CTC494 on the pathogen growth in surimi was temperature dependent, i.e., at 2 °C growth of *L. monocytogenes* slightly continued after the LAB strain reached its  $N_{max}$ ,

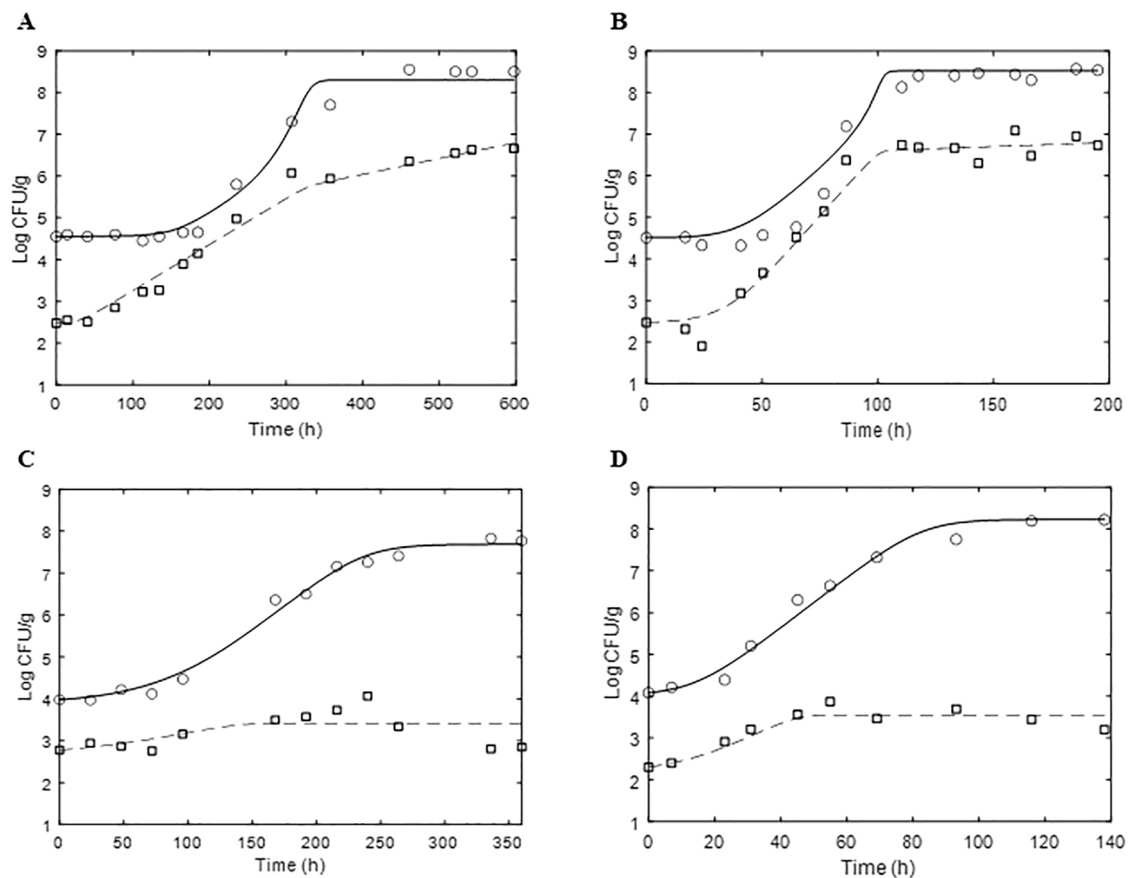


Fig. 2. Observed and predicted growth of *Lactilactobacillus sakei* CTC494 (○) and *Listeria monocytogenes* CTC1034 (□), in coculture, on sliced surimi product (A and B) and tuna pâté (C and D). A and C refer to temperatures of 2 °C and B and D refer to temperatures of 12 °C. Growth of *L. sakei* (solid line) and *L. monocytogenes* (dashed line) was described by the new expanded Jameson model of the present study (Eq. (4)).

whereas at 12 °C growth of *L. monocytogenes* stopped when *L. sakei* reached its  $N_{max}$ . The latest phenomenon corresponds to the classical Jameson-effect, which describes a non-specific interaction between microbial populations that are similarly inhibited by the depletion of the same nutrient or by the production of the same end-product (Giménez & Dalgaard, 2004; Jameson, 1962; Le Marc, Valík, & Medvedová, 2009).

The observations in sliced surimi at 2 °C (i.e., lag-reducing effect in coculture together with the reduced pathogen  $N_{max}$ ) suggest that at the early stages of the storage period the type of microbial interaction could be categorized as mutualistic since both interacting species gained fitness (Smid & Lacroix, 2013). This relationship gradually evolved into amensalism (0/- interaction) as *L. sakei* dominated the food system. Note that amensalism can be considered as a form of asymmetrical antagonistic (competitive) interaction between species (Mougi, 2016). These phenomena could be explained by the fact that microorganisms can lie on a continuum between mutualism and antagonism which can shift with environmental changes (i.e., catabolism of carbon sources, production of by-products, ...) (Roossinck, 2015). Thus, a mutualism-antagonism continuum could be hypothesized in sliced surimi at 2 °C. In this regard, Yang et al. (2017) evidenced a mutual growth-promoting effect between *Bifidobacterium bifidum* and *L. monocytogenes*, and its molecular mechanism was investigated. They observed that the expression of proteins related to biosynthesis and cell reproduction was upregulated after growth in coculture, promoting the growth of the two bacteria, which led to an earlier entry into the exponential growth phase. Acai, Valík, Medved'ová, and Roskopf (2016) reported a positive effect of *Escherichia coli* on the growth of a starter LAB culture in milk and mentioned that "the LAB generally requiring complex media were supported by the metabolites produced by *E. coli* population". These examples demonstrate the complex web of interactions between species in mixed cultures.

In tuna pâté, growth kinetics of *L. sakei* CTC494 in coculture were comparable to that observed in monoculture, reaching similar  $N_{max}$  values (7.69 log CFU/g). In contrast, the growth of *L. monocytogenes* was significantly affected by the presence of *L. sakei* CTC494 at both storage temperatures. As can be observed in Fig. 2C, the growth of the pathogen at 2 °C slightly increased up to 10 storage days, reaching maximum count values of  $4.07 \pm 0.12$  log CFU/g. At 12 °C (Fig. 2D), *L. sakei* CTC494 limited *L. monocytogenes* growth (<2 log increase) even before *L. sakei* approached to its  $N_{max}$ , which suggests a specific inhibition activity by the LAB strain rather than the classical Jameson-effect. In this

regard, *L. sakei* CTC494 is a low acidifying culture, according to the measured pH and lactic acid values (section 3.1); thus, the influence of by-products such as organic acids would be discarded as potential inhibitors. The pathogen suppression could be chiefly attributed to the bacteriocinogenic capacity of *L. sakei* CTC494, specifically, to the production of the antilisterial bacteriocin sakacin K (Hugas et al., 1995; Leroy, Lievens, & De Vuyst, 2005). In fact, Hugas, Pages, Garriga, and Monfort (1998) confirmed sakacin K production by *L. sakei* CTC494 in MRS broth at a range of temperatures from 4 °C to 30 °C and at initial pH from 5 to 6.5, reporting higher bacteriocin production at low temperatures (4 and 10 °C). Recent studies have reported different degrees of inhibition exerted by *L. sakei* CTC494 against *L. monocytogenes* in fish products. For instance, Aymerich et al. (2019) demonstrated the total suppression of the pathogen growth (<0.5 log increase) in cold-smoked salmon during storage at 8 °C, while Bolívar et al. (2021) stated the temperature dependency of the bioprotective efficacy in hot-smoked sea bream, i.e., by limiting (<2 log increase) or inhibiting (<1 log increase) the pathogen growth under different temperature conditions.

### 3.4. Modelling microbial interaction in RTE fish products

The competition coefficients estimated by the tested microbial interaction models are shown in Table 3. Fig. 2 presents the fitting with the new expanded Jameson-effect model (Eq. 4) for simultaneous growth of *L. sakei* CTC494 and *L. monocytogenes* in RTE fish products. In general, the expanded Jameson model (Eq. 3) could not sufficiently describe the competitive growth curves in the studied fish products. That is, this model showed higher RMSE and AICc values for simultaneous growth curves than that of the new expanded Jameson model (notice that the lower the values of RMSE and AICc, the better the model fit).

In surimi product, the interaction coefficient  $\gamma_{12}$  estimated by fitting the expanded Jameson model indicated no microbial interaction between *L. sakei* and *L. monocytogenes* at 2 °C ( $\gamma_{12} = 0$ ) while a  $\gamma_{12}$  value of ca. 1.0 was determined at 12 °C, describing the classical Jameson-effect. Notice that the  $\gamma$ -values estimated by the new expanded Jameson model should not be equally interpreted than the expanded Jameson model due to the use of additional competition parameters. Although the aforementioned  $\gamma_{12}$  values are in agreement with the observed competition pattern (see growth data in Fig. 2A and B), the expanded Jameson-effect model produced a poor representation of the experimental growth

**Table 3**

Interaction coefficients with 95% confidence intervals (lower limit, upper limit) as estimated by the expanded Jameson-effect model (Eq. 3) and the new expanded Jameson-effect model (Eq. 4) used to describe the simultaneous growth of *L. sakei* CTC494 and *L. monocytogenes* in ready-to-eat (RTE) fish products.

| Product       | T (°C) | Expanded Jameson model |  |                   |                   | New expanded Jameson model  |  |                           |                          |       |        |
|---------------|--------|------------------------|--|-------------------|-------------------|-----------------------------|--|---------------------------|--------------------------|-------|--------|
|               |        | $\gamma_{12}^a$        | $\gamma_{21}^b$  | RMSE <sup>c</sup> | AICc <sup>d</sup> | $\gamma_{12}$               | $\gamma_{21}$  | $\beta_{12}^e$            | $\beta_{21}^f$           | RMSE  | AICc   |
| Sliced surimi | 2      | 0.057<br>[-0.10, 0.25] | -3.527·10 <sup>5</sup><br>[-1.25·10 <sup>6</sup> , -1.85·10 <sup>4</sup> ] | 0.988             | 6.79              | 0.651<br>[0.36, 0.87]       | -1.797·10 <sup>3</sup><br>[-2.11·10 <sup>4</sup> , -5.10·10 <sup>2</sup> ] | -15.969<br>[-32.64, 0.90] | -0.034<br>[-1.06, -0.01] | 0.348 | -19.62 |
|               | 12     | 1.034<br>[0.92, 1.54]  | -18.531<br>[-169.93, 240.83]   | 0.866             | 1.26              | 0.968<br>[0.86, 1.63]       | -3.604·10 <sup>3</sup><br>[-2.84·10 <sup>3</sup> , 295.87] <sup>g</sup>    | 20.356<br>[-0.29, 27.50]  | 0.003<br>[0.00, 0.02]    | 0.557 | -7.32  |
| Tuna pâté     | 2      | 2.012<br>[1.57, 2.45]  | -2.374·10 <sup>3</sup><br>[-7.72·10 <sup>3</sup> , -733.92]                | 0.554             | -7.37             | 27.948<br>[-139.95, 113.89] | -6.402·10 <sup>3</sup><br>[-2.26·10 <sup>4</sup> , -3.48·10 <sup>3</sup> ] | 2.224<br>[-30.59, 27.24]  | 0.030<br>[0.00, 0.07]    | 0.454 | -3.84  |
|               | 12     | 1.395<br>[1.26, 1.58]  | -1.820·10 <sup>4</sup><br>[-6.48·10 <sup>4</sup> , -4.50·10 <sup>3</sup> ] | 0.696             | 0.52              | 0.001<br>[0.00, 0.00]       | -0.005<br>[-0.01, 0.00]  | -0.005<br>[-0.01, 0.00]   | 0.008<br>[0.00, 0.02]    | 0.343 | -1.49  |

<sup>a</sup> Competition coefficient describing the effect of *L. sakei* CTC494 on *L. monocytogenes* growth ( $\gamma_{12}$ ).

<sup>b</sup> Competition coefficient describing the effect of *L. monocytogenes* on *L. sakei* CTC494 growth ( $\gamma_{21}$ ).

<sup>c</sup> Root Mean Squared Error (RMSE).

<sup>d</sup> Corrected Akaike Information Criterion (AICc).

<sup>e</sup> Competition coefficient describing the effect of *L. sakei* CTC494 on the lag time of *L. monocytogenes* ( $\beta_{12}$ ).

<sup>f</sup> Competition coefficient describing the effect of *L. monocytogenes* on the lag time of *L. sakei* CTC494 ( $\beta_{21}$ ).

<sup>g</sup> The estimated value fell outside the 95% confidence interval probably as result of an asymmetric distribution of the parameter error.

curves in surimi product as evidenced by the goodness-of-fit measures RMSE and AICc. However, the new expanded Jameson model, that included an additional coefficient of interaction ( $\beta$ ), satisfactorily simulated the simultaneous growth of both microorganisms as demonstrated in Fig. 2, yielding much lower RMSE and AICc values (Table 3). In surimi product at 2 °C, the effect of microbial interaction on lag time was showcased by the estimated interaction coefficient values ( $\beta_{12}$  and  $\beta_{21} < 0$ ) indicating a  $\lambda$  reduction of both microorganisms and consequently a mutualistic effect between populations. The *L. monocytogenes* interaction coefficient  $\beta_{21}$  was ca. zero at 12 °C, indicating no effect by the pathogen on the  $\lambda$  of *L. sakei* CTC494.

In tuna pâté, both the existing Jameson and the new expanded Jameson models reflected the bacteriostatic/inhibitory effect of *L. sakei* CTC494 on *L. monocytogenes* growth, providing the latest slightly better fit according to the RMSE values (Table 3). In fact, *L. sakei* competition coefficient ( $\gamma_{12}$ ) fitted by expanded Jameson model was  $> 1$  at both storage temperatures, indicating that *L. monocytogenes* growth was inhibited by the LAB strain after reaching its  $N_{max}$ , as observed in Fig. 2C. In particular, the new expanded Jameson-effect equation appropriately described the pathogen growth inhibition ( $< 2$  log increase) at 12 °C as shown in Fig. 2D and RMSE value of 0.343. Indeed, the values of interaction coefficient ( $\beta$ ), close to zero, confirmed the convergence of both models since no strong interaction was observed for  $\lambda$  in this specific product.

Studies dealing with modelling of microbial competition in foods have been focussed on antagonistic interactions in which the main underlying mechanism is the consumption of the limiting substrate and/or the production of active inhibitory metabolites. This type of interspecies competition and its effect on the maximum population density ( $N_{max}$ ) has been successfully described by the classical Jameson-effect model or its variants in a widely range of foods (Cadavez et al., 2019; Cornu et al., 2011; Mejlholm & Dalgaard, 2015; Ye et al., 2014). In our study, the introduction of an additional parameter ( $\beta$ ) in an existing Jameson-effect model allowed for description of not only antagonism but also mutualism-based interactions based on their influence on lag time ( $\lambda$ ).

#### 4. Conclusion

This work made a first attempt to deep into the quantitative characterisation of microbial interaction considering the significance of different food matrices on the efficacy of bacteriocinogenic LAB cultures against *L. monocytogenes* by using microbial interaction models. The different competition patterns obtained in the tested fish products suggest that the inhibiting effect of *L. sakei* CTC494 on growth of *L. monocytogenes* was food product dependent. Interestingly, a mutualism-antagonism continuum of both populations was observed at 2 °C in surimi product with a mutualistic effect on lag time ( $\lambda$ ). The application of an additional interaction coefficient ( $\beta$ ) in the expanded Jameson-effect model offered the possibility to quantitatively characterize the different competition patterns. Further research based on omics approaches (e.g., proteomics, transcriptomics) should be applied to elucidate the underlying mechanisms of the different microbial interactions observed in the studied fish products.

#### CRedit authorship contribution statement

**Araceli Bolívar:** Methodology, Software, Validation, Writing - original draft, Visualization. **Fatih Tarlak:** Software, Investigation. **Jean Carlos Correia Peres Costa:** Conceptualization, Methodology, Investigation. **Manuel Cejudo-Gómez:** Investigation. **Sara Bover-Cid:** Investigation, Writing - review & editing. **Gonzalo Zurera:** Resources, Funding acquisition, Writing - review & editing. **Fernando Pérez-Rodríguez:** Conceptualization, Writing - review & editing, Software, Supervision.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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