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Modelling the interaction of the sakacin-producing *Lactobacillus sakei* CTC494 and *Listeria monocytogenes* in filleted gilthead sea bream (*Sparus aurata*) under modified atmosphere packaging at isothermal and non-isothermal conditions

Costa, Jean Carlos Correia Peres\(^a\), Bover-Cid, Sara\(^b\), Bolívar, Araceli\(^a\), Zurera, Gonzalo\(^a\), Pérez-Rodríguez, Fernando\(^a\)*.

\(^a\)Department of Food Science and Technology, Faculty of Veterinary, Agrifood Campus of International Excellence (CeiA3), University of Córdoba, Córdoba, Spain

\(^b\)IRTA, Food Safety Programme – Finca Camps i Armet s/n, 17121 Monells, Spain

*Corresponding author at:* Department of Food Science and Technology, University of Córdoba, Edif. Darwin-anexo (C1), Campus de Rabanales s/n, Crta, Madrid-Cádiz Km 396A, 14014, Córdoba, Spain.

*Email address:* b42perof@uco.es
Highlights

- *L. sakei* CTC494 inhibited *L. monocytogenes* growth in sea bream fillets during chilled and moderate abuse temperature storage.

- *L. sakei* CTC494 did not increase deterioration of filleted sea bream at an initial level of ≤ 4 log cfu/g.

- *L. sakei* CTC494 showed potential as bioprotective culture for fish products.

- An approach from broth to food was developed for modelling microbial interaction.

- Interaction models simulated *L. monocytogenes* inhibition by the bioprotective *L. sakei* in filleted sea bream under static and dynamic temperature.
The objective of this work was to quantitatively evaluate the effect of *Lactobacillus sakei* CTC494 (sakacin-producing bioprotective strain) against *Listeria monocytogenes* in fish juice and to apply and validate three microbial interaction models (Jameson, modified Jameson and Lotka Volterra models) through challenge tests with gilthead sea bream (*Sparus aurata*) fillets under modified atmosphere packaging stored at isothermal and non-isothermal conditions. *L. sakei* CTC494 inhibited *L. monocytogenes* growth when simultaneously present in the matrix (fish juice and fish fillets) at different inoculation ratios pathogen:bioprotector (i.e. 1:1, 1:2 and 1:3). The higher the inoculation ratio, the stronger the inhibition of *L. monocytogenes* growth, with the ratio 1:3 yielding no growth of the pathogen. The maximum population density (*N*\textsubscript{max}) was the most affected parameter for *L. monocytogenes* at all inoculation ratios. According to the microbiological and sensory analysis outcomes, an initial inoculation level of 4 log cfu/g for *L. sakei* CTC494 would be a suitable bioprotective strategy without compromising the sensory quality of the fish product. The performance of the tested interaction models was evaluated using the Acceptable Simulation Zone approach. The Lotka Volterra model showed slightly better fit than the Jameson-based models with 75-92 % out of the observed counts falling into the Acceptable Simulation Zone, indicating a satisfactory model performance. The evaluated interaction models could be used as predictive modelling tool to simulate the simultaneous behaviour of bacteriocin-producing *Lactobacillus* strains and *L. monocytogenes*; thus, supporting the design and optimization of bioprotective culture-based strategies against *L. monocytogenes* in minimally processed fish products.
Keywords: biopreservation, food-borne pathogen, lactic acid bacteria, competition model, minimally processed fish, predictive microbiology
1. Introduction

Global consumption of fresh and minimally processed fish has grown rapidly in recent decades. In this regard, aquaculture has been responsible for the extraordinary growth in the supply of fish for human consumption, which resulted in a record-high per capita consumption of 20.3 kg in 2016 (FAO, 2018). The combination of chemical oxidation of lipids, autolytic biochemical reactions and physico-chemical characteristics make fish a highly perishable product, but also an ideal environment for growth of spoilage microorganisms and food-borne pathogens (Dalgaard et al., 2006; Parlapani et al., 2014). Among the pathogenic bacteria, *Listeria monocytogenes* stands out because of its ability to tolerate salty environments and multiply in refrigerated foods, coupled with the high mortality rates in humans (CDC, 2017). The pathogen has been isolated from a variety of raw fish and processed fish products (Abdollahzadeh et al., 2016; Lennox et al., 2017; Rožman et al., 2016), and according to the last report of the European Food Safety Authority (EFSA), “fish and fishery products” showed the highest levels of non-compliance with the food safety microbiological criteria for *L. monocytogenes* laid down by Regulation (CE) 2073/2005 (EFSA, 2017).

Lactic acid bacteria (LAB), and lactobacillus in particular, constitute the dominant microbiota in several types of foods and many LAB species are used as microbial food cultures (MFC) in food production. In the EU, there is no specific regulation regarding MFC; but with a long history of safe use, they are considered traditional food ingredients and are legally permitted without premarket approval. Thus, MFC defined as characteristic food ingredients must be listed on the ingredient labels of the final food in agreement with the Regulation (EU) 1169/2011. In addition, when added to a food, MFC must comply with the requirements established in the General Food Law
(Regulation (EC) 178/2002), i.e. they must be safe for their intended use (Herody et al., 2010; Laulund et al., 2017). Many LAB genera and species are generally recognized as safe (GRAS) by the FDA (2018) and have the qualified presumption of safety (QPS) status established by EFSA. Among LAB, *Lactobacillus* is the genus including a high number of GRAS species, and particularly, *Lactobacillus sakei* is included in the QPS list (EFSA BIOHAZ, 2017), thus not requiring the full safety assessment (antibioresistance, virulence, and biogenic amine characterization) for its market authorisation in the EU. The application of selected LAB strains as bioprotective cultures has demonstrated a high potential to inhibit undesirable spoilage and pathogenic bacteria in fresh fish and RTE fish products, including *L. monocytogenes* (Anacarso et al., 2014; Brillet et al., 2005). The inhibitory mechanism of LAB includes microbial growth competition as well as microbial antagonism associated with the production of antimicrobial metabolites such as organic acids (lactic acid, acetic acid, etc.), hydrogen peroxide and more specifically, bacteriocins active against specific bacteria such as *L. monocytogenes* (Gómez-Sala et al., 2016). In relation to the latter, sakacins, being produced by certain *L. sakei* strains, belong to subclass IIa of bacteriocins which are generally known to have a strong antilisterial activity (Leroy and De Vuyst, 2000). The lethal action of these bacteriocins results from membrane pore formation of the target cell causing depletion of vital components as well as dissipation of the proton motive force (Héchard and Sahl, 2002).

Microbial interaction has been addressed in the predictive microbiology field mainly focused on the inhibitory effect of endogenous LAB on *L. monocytogenes* behavior (Mejlholm and Dalgaard, 2007). Interaction models are usually intended to quantify how much the growth of one population is reduced by the growth of other populations.
Thus, two model approaches are generally used to describe the interaction of LAB and *L. monocytogenes*: i) those based on the Jameson effect phenomenon (Jameson, 1962) that describes the simultaneous stop of growth of all bacterial populations at the time when the dominant bacteria population reaches its stationary phase (Giménez and Dalgaard, 2004; Mellefont et al., 2008; Møller et al., 2013) and ii) the predator-prey models based on the Lotka Volterra equation, which allow to describe the dynamics of two competing bacterial populations by incorporating an additional term describing the reduction of the growth rate of a given population, this being proportional to the population density of other competing population (Powell et al., 2004; Valenti et al., 2013; Vereecken et al., 2000).

Predictive models dealing with the interaction between the pathogen *Listeria* and bacteriocin-producing LAB strains in foods other than fermented meat products (Drosinos et al., 2006; Leroy et al., 2005) are, to the best knowledge of the authors, not available in literature. Their development would provide the food industry with valuable tools to evaluate the effect of potential bioprotective cultures against *L. monocytogenes* in specific food matrices, thereby enhancing food safety. In this respect, minimally processed and RTE fish products made of raw fish, which are consumed without applying any lethal treatment, could pose a serious risk in relation to *L. monocytogenes* (Jami et al., 2014; Miettinen and Wirtanen, 2005; Rožman et al., 2016). Sea bream, considered a valuable fish species in Mediterranean EU countries, has been included over the last years as main ingredient in popular non-heated RTE fish products, such as sushi, carpaccio and other products (Bolívar et al., 2018). This fish species is mostly
commercialized fresh as whole fish and in several supermarket chains as filleted fish under modified atmosphere packaging (MAP).

Therefore, the objective of this work was i) to quantitatively evaluate the effect of the sakacin-producing bioprotective strain *Lactobacillus sakei* CTC494 against *L. monocytogenes* in a fish model system and ii) to apply and validate microbial interaction models to simulate the simultaneous growth of both microorganisms in gilthead sea bream (*Sparus aurata*) fillets under MAP at isothermal and non-isothermal conditions.

2. Material and Methods

2.1. A step-wise approach for interaction model development

A step-wise approach was followed to develop interaction models simulating the growth of the bioprotective *L. sakei* CTC494 and *L. monocytogenes* CTC1034 in fish fillets under MAP during isothermal and dynamic storage temperature. A schematic overview of the step-wise method is shown in Figure 1.

In the first step, the primary kinetic parameters lag time ($\lambda$), maximum specific growth rate ($\mu_{\text{max}}$) and maximum population density ($N_{\text{max}}$) were obtained for each microorganism from experimental data in mono-culture in fish (sea bream) juice at different temperature conditions (section 2.3.1) and based on those, secondary models were generated (section 2.7.1 and 2.7.2). Secondly, experimental data obtained in fish juice in co-culture were used (section 2.3.2) to estimate competition parameters in interaction models by means of a regression process (section 2.7.4). In a third step, the parameters from the secondary models and estimated interaction parameters for the model showing the best performance were used to simulate microbial interaction on fish
fillets stored under MAP at isothermal and non-isothermal conditions (section 2.5). The values for interaction parameters were assumed to be constant in the tested ratios for both microorganisms, hence the average from all assayed temperatures was used to define these parameters. Since an effect of the fish matrix and MAP conditions on kinetic parameters was expected, the specific growth rate obtained in fish juice was adjusted to consider such effects. To determine the adjustment factor, data from experiments made with fish fillets (section 2.5) were used, in which both microorganisms were inoculated at the same level and monitored under the same temperature conditions used in the fish juice experiments. The adjustment factor for $\mu_{\text{max}}$ of each microorganism was calculated as the ratio between the $\mu_{\text{max}}$ values obtained in fish product and in fish juice and were assumed to be constant for the range of temperatures tested. Therefore, the same adjustment factor was applied to simulate the microbial interaction on fresh fish fillets at isothermal and dynamic temperature conditions.

### 2.2. Bacterial strains and inoculum preparation

The bacteriocin-producing *L. sakei* CTC494 strain was selected as bioprotective culture in this study. This strain is a producer of bacteriocin, sakacin K, being able to inhibit the growth of spoilage bacteria and *Listeria* (Hugas et al., 1993). The strain *L. monocytogenes* CTC1034 previously used as indicator to study the antagonism the LAB produced bacteriocins (Garriga et al., 2002) was used in the present study as target pathogen. This strain has the same serotype (i.e. 4b) as the clinical isolate Scott A. Stock cultures were stored at -80 °C in de Man Rogosa and Sharpe (MRS, Oxoid, UK) broth for the LAB strain and in Brain Heart Infusion (BHI, Oxoid) for the pathogen, both with 20% glycerol as cryoprotectant.
Before experiments, *L. sakei* CTC494 and *L. monocytogenes* CTC1034 were pre-cultured separately at static conditions in MRS (Oxoid, UK) at 33 °C with 10 % CO₂ and BHI broth (BHI, Oxoid) at 37 °C, respectively. Two consecutive 24 h-subcultures were made for each microorganism by transferring 0.1 mL to tubes containing 9 mL of fresh respective media and incubating at the same above-mentioned temperatures. Then, a third subculture was prepared, and tubes were incubated for 18-20 h at the appropriate temperature resulting 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.

2.3. Experiments with *L. sakei* CTC494 and *L. monocytogenes* CTC1034 in mono- and co-culture in fish juice

Sterile fish juice was prepared from fresh muscle of gilthead sea bream following the protocol described by Bolívar et al. (2018). The prepared cultures (Section 2.2) were twice-washed in phosphate buffered saline solution (PBS) (Medicago AB, Uppsala, Sweden) by centrifugation at 4100 rpm (Jouan C4i, Thermo Electron Corporation, France) for 10 min and cells were re-suspended in fish juice. The suspensions of *L. monocytogenes* and *L. sakei* were serially diluted ten-fold in fish juice to obtain the desired concentration to be inoculated to fish juice at 1 % (v/v).

Growth experiments were carried out at static conditions in sterile 250-mL Schott bottles containing fish juice. In the mono-culture experiments, the inoculum concentration of each microorganism was set to ca. $10^2$ cfu/mL. For the co-culture experiments, the inoculum concentration of *L. monocytogenes* was always $10^2$ cfu/mL, while for *L. sakei* CTC494, three different concentrations were investigated, ($10^2$, $10^4$ and $10^6$ cfu/mL), thus generating three (initial) inoculation ratios *L. monocytogenes*: *L.*
sakei that corresponded to 1:1, 1:2 and 1:3 when bacterial concentrations were expressed in logarithmic scale. After inoculation, flasks were stored at four constant temperatures targeted at 2, 5, 8 and 12 °C during a period from 5 to 46 days. Storage temperature was recorded at regular time intervals using data loggers (Fourtec, MiniLitE5032L, USA) and the mean of registered temperatures (i.e. 2.2, 5.0, 8.1 and 12.1 °C) was used for modelling purposes. Each experiment was performed in duplicate.

2.4. Quality deterioration assessment of fresh sea bream fillets under MAP

2.4.1. Fish fillet product description

Individual plastic trays containing two fresh gilthead sea bream fillets packed under MAP were supplied by a private company (Zaragoza, Spain). Fish trays were received at the laboratory 18-24 h after processing in expanded polystyrene boxes with flake ice. The average weight of the fish fillets was 332.2 ± 12.1 g with an initial pH of 6.11 ± 0.05 (Hanna Edge, HI2020, USA). The initial headspace gas composition in the trays was measured using a O₂/CO₂ gas analyser (Gaspace 2, Systech Instruments, U.K.) and the obtained values corresponded to 37.4 % ± 0.7 for O₂ and 27.0 % ± 1.0 for CO₂.

2.4.2. Inoculation of fish fillets

Bacterial suspensions prepared as described in Section 2.2 were serially diluted ten-fold with physiological saline water (PSW, 0.85 % w/v NaCl). For inoculation, aliquots of 0.01 mL were taken from the appropriate decimal dilution and deposited on the caudal region of the fish fillet. Inoculation was performed using a 1-mL syringe with needle (BD Plastipak, Spain) inserted through an adhesive septum (Ø 15 mm, PBI Dansensor, Denmark) which was previously placed on the laminate film of the plastic tray.
2.4.3. Sensory analysis

A preliminary sensory analysis was conducted to assess the effect of the initial level of *L. sakei* CTC494 on fish quality deterioration. In that aim, fish fillets were inoculated with *L. sakei* CTC494 as described in the previous section at three initial concentrations of $10^2$, $10^4$ and $10^6$ cfu/g ($n = 14$, 14 and 10, respectively). A control batch was prepared without added bacteria ($n = 14$). All trays were stored at $5.0 \pm 0.12 ^\circ C$.

A semi-trained sensory panel made up of five members from the Faculty of Veterinary (University of Cordoba, Spain) was required in order to evaluate the quality changes of the fish fillets using the Quality Index Method (QIM) (Bremner et al., 1985). This method is based on the use of significant sensory parameters and characteristic attributes for raw fish with a scoring system of demerit points ($\leq 3$), which is in direct proportion to their importance in the deterioration pattern of the species (Huidobro et al., 2000). The scores for all the characteristics are summed-up to give an overall sensory score, the so-called Quality Index (QI) (Botta 1995). A QI of 0 indicates a very fresh fish and score increases as the freshness’s characteristics lapses (Campus et al., 2011).

In our study, the QIM was adapted from the scheme proposed by Lougovois et al. (2003) and Campus et al. (2011) to evaluate freshness in gilthead sea bream fillets under MAP. The attributes scored by the sensory panel are shown in Supplementary Table S1. A linear correlation was established for each experimental condition (i.e. control and inoculated batches) between the freshness expressed by the QI and storage time (Microsoft Excel, Redmond, USA). The QI scores obtained by the five panellists in each evaluation day for inoculated and control fillets were statistically compared by a t-
Student test ($p=0.05$) using the statistical software package SPSS 24.0 (Chicago, Illinois, USA).

Sensory results demonstrated that the rate of freshness loss was similar for fillets inoculated with $10^2$ and $10^4$ cfu/g of *L. sakei* compared to control fillets (data not shown). Hence, a level of $10^2$ and $10^4$ cfu/g of *L. monocytogenes* and *L. sakei*, respectively (ratio 1:2 in log scale) was defined for co-inoculation experiments in fish fillets (Section 2.5.2).

The application of *L. sakei* CTC494 as protective culture was sensory validated on fish fillets inoculated with both microorganisms at a ratio 1:2, which corresponded to, in arithmetic scale, ca. $10^2$ cfu/g *L. monocytogenes* CTC1034 and ca. $10^4$ cfu/g *L. sakei* CTC494. Inoculated fish and control (i.e. non-inoculated) fillets were stored at 5 °C for 8 days. Sensory assessment was performed on days 0, 4, 6 and 8.

### 2.5. Experiments with *L. sakei* CTC494 and *L. monocytogenes* CTC1034 on fresh gilthead sea bream fillets

#### 2.5.1. Mono-culture experiments

The effect of food matrix on the growth of *L. sakei* CTC494 and *L. monocytogenes* CTC1034 was evaluated by inoculating both microorganisms independently in fresh fish fillets. For that, fish fillets were acquired and inoculated ($n=36$) as described in Sections 2.4.1 and 2.4.2. An additional control batch ($n=22$) with non-inoculated fish fillets was prepared. Experiments were carried out at a target temperature of 5 °C (measured mean temperature of 4.8 °C) for 25 days until microorganisms reached the stationary phase.
2.5.2. Co-culture experiments

The interaction between *L. sakei* and *L. monocytogenes* on fish fillets was evaluated by co-inoculation at the selected 1:2 ratio (i.e. 2 log cfu/g *L. monocytogenes* and 4 log cfu/g *L. sakei*), which was previously defined according to results from Section 2.4.3. Before inoculation, bacterial suspensions were serially diluted ten-fold in PSW to obtain the desired concentration and mixed at equal volumes. Control (*n* = 56) and inoculated (*n* = 106) fillets were stored at two isothermal conditions with a mean of 4.8±0.14 and 8.2±0.10 °C for to 14 and 10 days, respectively. For the experiments at non-isothermal conditions, fillets were stored at two dynamic temperature profiles, ranging from 4 to 8 °C (profile 1) and from 2.5 to 12 °C (profile 2), for a total period of 12 days. The storage temperature was recorded at regular time intervals using data loggers (Fourtec, MiniLitE5032L, USA).

2.6. Microbiological analyses

For experiments in fish juice, at each sampling point, 1 mL sample was aseptically taken from each flask and serially diluted ten-fold in PSW. For experiments with fish product, a 25-g portion of the (inoculated) fish fillet’s caudal region, considered as the analytical sample, was taken aseptically and transferred to a stomacher bag containing 225 mL PSW. Samples were homogenized for 60 s (1500 rpm) in a stomacher (Masticator, IUL Instruments, Spain).

MRS agar supplemented with bromocresol purple (BP, 0.12 g/L, Sigma-Aldrich, USA) and *Listeria* selective agar base (Oxoid) containing selective supplement (SR140E; Oxoid) were used for the enumeration of *L. sakei* and *L. monocytogenes*, respectively. 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 et al., 2012). Plates were incubated for approx. 48 h at 33 °C under 10% CO2 for L. sakei and at 37 °C for L. monocytogenes.

2.7. Development of predictive models

2.7.1. Primary model fitting to mono-culture data

Plate counts for L. sakei and L. monocytogenes were transformed into decimal logarithmic values (i.e. log cfu/g or mL). The growth parameters λ, μ_{max} and N_{max} obtained from each storage temperature for mono and co-culture experiments were estimated by fitting the Baranyi and Roberts model (1994) defined by Eqs. (1) and (2) to the observed data (mean of duplicates at each sampling point) using DMFit Excel Add-in v. 3.5.

\[
\log N_t = \log N_0 + \frac{\mu_{max}}{\ln(10)} \cdot F(t) - \frac{1}{m \cdot \ln(10)} \cdot \ln \left(1 + \frac{e^{m \cdot \mu_{max} \cdot F(t)} - 1}{10^{m\log N_{max} - \log N_0}}\right) \quad (1)
\]

\[
F(t) = t - \lambda + \frac{1}{\mu_{max}} \cdot \ln \left(1 - e^{-\mu_{max} \cdot t} + e^{-\mu_{max} \cdot (t-1)}\right) \quad (2)
\]

where \(N_t\) is the cellular concentration (cfu/g or mL) at time \(t\), \(N_0\) is the initial concentration (cfu/g or mL), \(\mu_{max}\) is the specific maximum growth rate (h^{-1}), \(\lambda\) is the lag time (h), \(N_{max}\) is the maximum population density (cfu/g or mL), \(m\) is a curvature factor, \(F(t)\) represents an adjustment function for the model.

2.7.2. Secondary models for mono-culture experiments
The influence of temperature on the primary growth parameters of *L. sakei* and *L. monocytogenes* in fish juice was estimated using the square-root model (Eq. 3) (Ratkowsky et al., 1982) which was fitted in MS-Excel (Microsoft, Redmond, USA).

\[ \sqrt{p} = b \cdot (T - T_{\text{min}}) \]  

where \( p \) is the kinetic parameter (i.e. \( \lambda \) and \( \mu_{\text{max}} \)), \( b \) is a constant, \( T \) (°C) is temperature and \( T_{\text{min}} \) is the theoretical minimum temperature for growth.

### 2.7.3. Effect of microbial interaction on kinetics parameters

To quantify the reduction on *L. monocytogenes* growth by the bioprotective *L. sakei* CTC494 in fish juice, a reduction ratio \( (\alpha) \) was calculated based on the fraction between the parameters obtained in co-culture \( (p_{\text{co}}) \) and mono-culture \( (p_{\text{mono}}) \) as shown by Eq. (4). To that aim, the parameters from co-culture experiments were also obtained by the Baranyi model (see section 2.7.1).

\[ \alpha = 1 - \left( \frac{p_{\text{co}}}{p_{\text{mono}}} \right) \]  

where \( \alpha \) is the reduction ratio and \( p_{\text{co}} \) and \( p_{\text{mono}} \) the kinetic parameters (i.e. \( \lambda \) and \( \mu_{\text{max}} \)) in co-culture and mono-culture, respectively.

### 2.7.4. Modelling microbial interaction between *L. sakei* CTC494 and *L. monocytogenes* CTC1034

To predict the simultaneous growth between the bioprotective *L. sakei* strain (at different initial concentrations) and *L. monocytogenes* in fish juice stored at 2.2±0.08, 5.0±0.33, 8.1±0.33 and 12.1±0.12 °C, three different microbial interactions models were tested.
Firstly, the Jameson effect model based on Eqs. (5) and (6), which assumes that the growth of the pathogen halts when the dominant microbial population reaches its $N_{max}$ (Cornu et al., 2011; Jameson, 1962).

$$\frac{dN_{ls}}{dt} = N_{ls} \cdot \mu_{max,ls} \cdot \left(1 - \frac{N_{ls}}{N_{max,ls}}\right) \cdot \left(1 - \frac{N_{lm}}{N_{max,lm}}\right) \cdot \left(\frac{Q_{ls}}{1+Q_{ls}}\right)$$  (5)

$$\frac{dN_{lm}}{dt} = N_{lm} \cdot \mu_{max,lm} \cdot \left(1 - \frac{N_{lm}}{N_{max,lm}}\right) \cdot \left(1 - \frac{N_{ls}}{N_{max,ls}}\right) \cdot \left(\frac{Q_{lm}}{1+Q_{lm}}\right)$$  (6)

$$\frac{dQ_{ls}}{dt} = Q_{ls-1} \cdot \mu_{max,ls}$$  (7)

$$\frac{dQ_{lm}}{dt} = Q_{lm-1} \cdot \mu_{max,lm}$$  (8)

where $N$ is the cell concentration (cfu/mL) at time $t$, $\mu_{max}$ is the maximum specific growth rate ($h^{-1}$), $N_{max}$ is the maximum population density (cfu/mL) and $Q$ is a measure of the physiological state of cells at time $t$, for L. sakei (Ls) or L. monocytogenes (Lm).

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

$$Q_0 = \frac{1}{e^{(\mu_{max,ls})} - 1}$$  (9)

In our study, a modification of the Jameson effect model was also used, represented by Eqs. (10) and (11). This modification includes the parameters $N_{cri,ls}$ and $N_{cri,lm}$ that describe the maximum critical concentration that a population should reach to inhibit the growth of the other population (Jameson, 1962; Le Marc et al., 2009; Vasilopoulos et al., 2010).
\[
\frac{dN_{Ls}}{dt} = N_{Ls} \cdot \mu_{\text{max} Ls} \cdot \left( 1 - \frac{N_{Ls}}{N_{\text{max} Ls}} \right) \cdot \left( 1 - \frac{N_{Lm}}{N_{\text{cri} Lm}} \right) \cdot \left( \frac{Q_{Ls}}{1 + Q_{Ls}} \right) \quad (10)
\]

\[
\frac{dN_{Lm}}{dt} = N_{Lm} \cdot \mu_{\text{max} Lm} \cdot \left( 1 - \frac{N_{Lm}}{N_{\text{max} Lm}} \right) \cdot \left( 1 - \frac{N_{Ls}}{N_{\text{cri} Ls}} \right) \cdot \left( \frac{Q_{Lm}}{1 + Q_{Lm}} \right) \quad (11)
\]

\[
\frac{dQ_{Ls}}{dt} = Q_{Ls-1} \cdot \mu_{\text{max} Ls}
\]

\[
\frac{dQ_{Lm}}{dt} = Q_{Lm-1} \cdot \mu_{\text{max} Lm}
\]

where \( N_{\text{cri}} \) is the maximum critical concentration (cfu/mL) of \( L. \text{sakei} \) (Ls) on \( L. \text{monocytogenes} \) (Lm) and vice-versa. The rest of model parameters are described in Eqs. (5) to (9).

Finally, the traditional Lotka Volterra model, also referred to as predator-prey model, was used according to Eqs. (14) and (15). This model includes two empirical parameters reflecting the degree of interaction between microbial species \( (F_{LsLm} \text{ and } F_{LmLs}) \) (Cornu et al., 2011; Fujikawa et al., 2014; Giuffrida et al., 2008). Depending on the empirical parameter value for \( L. \text{sakei} \) \( (F_{LsLm}) \), the growth of \( L. \text{monocytogenes} \) can be affected in three different ways:

1) If \( 0 < F_{LsLm} < 1 \), \( L. \text{monocytogenes} \) grows with reduced \( \mu_{\text{max}} \) after \( L. \text{sakei} \) reaches \( N_{\text{max}} \).

2) If \( F_{LsLm} = 1 \), \( L. \text{monocytogenes} \) stops growing when \( L. \text{sakei} \) reaches its \( N_{\text{max}} \).

3) If \( F_{LsLm} > 1 \), \( L. \text{monocytogenes} \) population declines when \( L. \text{sakei} \) reaches its \( N_{\text{max}} \).

\[
\frac{dN_{Ls}}{dt} = N_{Ls} \cdot \mu_{\text{max} Ls} \cdot \left( 1 - \frac{N_{Ls} + F_{LsLm} \cdot N_{Lm}}{N_{\text{max} Ls}} \right) \cdot \left( \frac{Q_{Ls}}{1 + Q_{Ls}} \right) \quad (14)
\]
\[
\frac{dN_{Lm}}{dt} = N_{Lm} \cdot \mu_{maxLm} \cdot \left(1 - \frac{N_{Lm} + F_{LmLs} \cdot N_{Ls}}{N_{maxLm}}\right) \cdot \left(\frac{Q_{Lm}}{1 + Q_{Lm}}\right)
\]

(15)

\[
\frac{dQ_{Ls}}{dt} = Q_{Ls-1} \cdot \mu_{maxLs}
\]

(16)

\[
\frac{dQ_{Lm}}{dt} = Q_{Lm-1} \cdot \mu_{maxLm}
\]

(17)

where \(F_{LdLm}\) and \(F_{LmLs}\) are, respectively, the competition factor parameters of \(L.\) sakei CTC494 on \(L.\) monocytogenes CTC1034 and vice-versa. The other parameters are as indicated in Eqs. (5-9).

The interaction parameters \(N_{cri}\) (maximum critical concentration of one population) and \(F_{LdLm}\) and \(F_{LmLs}\) (competition factors of one species on the other) were estimated by regression using kinetic parameters derived from mono-culture data (see sections 2.7.1 and 2.7.2). To estimate the best-fit values of interaction parameters, an optimization procedure was implemented in MATLAB version R2015b using the functions \textit{fmincon} and \textit{ode45}\ (The MathWorksInc®, Natick, USA).

2.7.5. \textit{Goodness-of-fit indexes and predictive model performance}

The goodness-of-fit of the primary and secondary models was assessed by root mean square error (RMSE) and coefficient of determination (\(R^2\)).

The performance of the developed interaction models to predict the behaviour of the bioprotective \(L.\) sakei CTC494 and \(L.\) monocytogenes CTC1034 in MAP-fish fillets under isothermal and dynamic temperature conditions was evaluated by the acceptable simulation zone (ASZ) approach. Model performance is considered acceptable when at least 70 % of the observed log counts values are within the ASZ, defined as ± 0.5 log-
units from the simulated concentration in log units (Mejlholm and Dalgaard, 2015; Møller et al., 2013).

3. Results

3.1. Primary growth parameters of L. sakei CTC494 and L. monocytogenes CTC1034 in mono-culture on fish juice and fish fillets

The two studied microorganisms were able to grow in sterile fish juice when stored at 2.2±0.08, 5.0±0.33, 8.1±0.33 and 12.1±0.12 °C and on fish fillets at 4.8±0.14 °C. The growth curves obtained from the fit of the Baranyi and Roberts model provided a good description of the observed data (Supplementary Figure S1). The parameters $\lambda$ and $\mu_{\text{max}}$ varied with temperature, while $N_{\text{max}}$ was not affected, with average values of 7.92 and 8.74 log cfu/mL for L. sakei CTC494 and L. monocytogenes, respectively. The parameters estimated by the Baranyi and Roberts model are shown in Table 1. For both fish matrices (juice and fillets) the model showed good fit to data ($R^2 > 0.98$) (Supplementary Table S2). A minimum of 7 and a maximum of 23 sampling points were taken for each microorganism depending on the storage temperature. In summary, results in mono-culture confirmed that the bioprotective strain L. sakei CTC494 presented better ability to grow in fish juice at low temperatures, which was also observed on fish fillets.

3.2. Secondary growth models

The parameters $\lambda$ and $\mu_{\text{max}}$ obtained from the Baranyi and Roberts model were used to fit a square-root model (Eq. 3). The ability of the secondary models to describe the influence of temperature on the growth parameters was proven to be satisfactory according to the values from RMSE and $R^2$, whose values were in the ranges 0.064-
0.086 and 0.874-0.999, respectively. A summary of results from the fitting of the square-root model for both microorganisms is shown in Table 2.

### 3.3. Interaction of L. sakei CTC494 and L. monocytogenes CTC1034 in fish juice at different temperatures and inoculation ratios

The influence of storage temperature and the inoculation ratio (1:1, 1:2 and 1:3) on the interaction of *L. sakei* CTC494 and *L. monocytogenes* was assessed. To allow a comparison with kinetic parameters in mono-culture, the Baranyi and Roberts model without considering interaction was fitted to experimental data in co-culture (Table 3). The statistical indexes for the fitted model presented satisfactory RMSE and $R^2$ values (Supplementary Table 3). The most evident outcome from these experiments was that higher ratios produced stronger inhibition of *L. monocytogenes* growth, with the ratio 1:3 yielding no apparent growth for the pathogen. The parameter $\mu_{max}$ was little influenced, even though values obtained in co-culture were generally lower than those obtained in mono-culture. Figure 2 represents, through a bar diagram, a comparison of $\lambda$ and $N_{max}$ obtained from mono-culture and co-culture at the different conditions by using the reduction ratio ($\alpha$) calculated according to Eq. (4). From this figure, it can be observed that $\alpha$ for $\lambda$ varied for *L. monocytogenes* among the different inoculation ratios, but in all co-culture experiments, $\lambda$ presented a reduction with respect to that observed in mono-culture. However, further analysis of data confirmed that differences were rather produced by the fitting process (i.e. prediction error) affected by the relatively $\lambda$ short duration ($\geq 5 \, ^\circ C; \lambda \leq 36 \, h$) than a hypothetical interaction between microorganisms.

On the other hand, $N_{max}$ was the most affected parameter for *L. monocytogenes* at all concentration ratios. For instance, in mono-culture experiments at 5.0 °C (Table 1), log
\( N_{\text{max}} \) was 8.65 log cfu/mL while for co-culture experiments, the parameter was gradually decreasing to 5.94 (\( \alpha = 31 \% \)), 4.22 (\( \alpha = 51 \% \)) and 1.37 (\( \alpha = 84 \% \)) log cfu/mL for inoculation ratios 1:1, 1:2 and 1:3, respectively. For the latter, the putative “\( N_{\text{max}} \)” was taken from observations since the Baranyi and Roberts model could not be fitted to data at ratio 1:3 as no growth was observed. Similar inhibition patterns were observed for the other assayed temperatures (Fig. 2).

3.4. Sensory analysis

The sensory evaluation results obtained for sea bream fillets under MAP conditions stored at 5 °C are presented in Table 4. The QI scores obtained for fish samples inoculated at a ratio 1:2 (\( L. \) monocytogenes: \( L. \) sakei) were compared to control samples (i.e. non-inoculated). In general, QI scores increased linearly during storage with a correlation coefficient (\( R^2 \)) of 0.82 and 0.67 for control and inoculated batches, respectively. The statistical analysis of QI scores showed that \( L. \) sakei CTC494 did not significantly affect the sensory properties of fish fillets (p > 0.05) during the evaluated storage time (8 days). Though the deterioration rate was slightly lower for control (slope = 0.47) than for inoculated samples (slope = 0.55), the differences were not statistically significant (p > 0.05). Therefore, from the sensory perspective, the addition level of \( 10^4 \) cfu/g of \( L. \) sakei CTC494 would be suitable as bioprotective strategy without modifying the spoilage rate in comparison with a control (non-bioprotected) product.

3.5. Modelling interaction of \( L. \) sakei CTC494 and \( L. \) monocytogenes CTC1034 in fish juice

The three interaction models (Fig. 3) were tested using the kinetic parameters (\( \lambda, \mu_{\text{max}} \) and \( N_{\text{max}} \)) obtained from the Baranyi and Roberts model fitted to mono-culture
experiment data and estimating the respective interaction factors by regression analysis. The statistical performance of the models was evaluated by RMSE whose values are shown in Table 5 together with the estimated parameters. The Jameson effect model presented the worst fitting to data, showing the highest RMSE values. This result suggests that interaction between both microorganisms could not be exclusively explained by the Jameson effect, where growth inhibition is the result from a depletion in nutrient bioavailability and toxicity increase when the dominant population reaches $N_{\text{max}}$. The modified Jameson effect model including the parameter $N_{\text{cri}}$ showed better performance, with RMSE lower values. For both microorganisms, $N_{\text{cri}}$ remained in the same order of magnitude for the different temperatures and inoculation ratios, with average values, in log scale, of 7.7 and 8 log cfu/mL for *L. sakei* CTC494 and *L. monocytogenes* CTC1034, respectively (Table 5). The Lotka Volterra interaction model showed slightly better fit to data than the above models according to RMSE (Table 5) and visual analysis of growth curves (Fig. 3). In the case of the ratio 1:3, a poor fitting was observed for *L. monocytogenes* although this condition also yielded unsatisfactory fitting results for the Jameson effect-based models. This could be due to the difficulty of the models to suitably describe the large decline of *L. monocytogenes* population at this ratio. As regards the inhibitory effect of *L. sakei* CTC494 on *L. monocytogenes* growth, competition factors ($F_{LsLn}$) at the lowest temperature (2.2 °C) were below 1 for inoculation ratios 1:1 and 1:2, with values of 0.84 and 0.94, respectively. However, for the same temperature at ratio 1:3, the competition factor was equal to 2.67. This higher value reflected the noticeable decline of *L. monocytogenes* population (down to 0.70 log cfu/mL). For inoculation ratios 1:2 and 1:3, at 5 °C, the competition factors increased up
to 1.54 and 1.46, respectively. For higher temperatures (8-12 ºC), this increasing trend in the competition factor was minimized showing a rather variable pattern, and therefore, no mathematical model could be derived for such a relationship. Thus, for modelling purposes, this parameter was fixed to the average value observed at different temperatures for the corresponding inoculation ratio.

*L. monocytogenes* did not exert any inhibitory effect on *L. sakei* CTC494 as demonstrated by the competition factor ($F_{LmLs}$) being equal to 0 (Table 5).

### 3.6. Simulation of growth interaction of *L. sakei* CTC494 and *L. monocytogenes* CTC1034 on fish fillets

The simultaneous growth of *L. sakei* CTC494 and *L. monocytogenes* was evaluated in sea bream fillets under MAP at two isothermal (4.8 and 8.2 ºC) as well as at two non-isothermal conditions (profile 1: 4-8 ºC and profile 2: 2.5-12 ºC) at an initial inoculation ratio 1:2 based on the sensory analysis outcome (Fig. 4).

For isothermal conditions, *L. sakei* CTC494 reached the stationary phase with 10 and 5 days of storage at 4.8 ºC and 8.2 ºC, respectively, with an average log $N_{\text{max}}$ of 7.91 log cfu/g, while for *L. monocytogenes* under the same conditions, log $N_{\text{max}}$ was 2.23 and 1.87 log cfu/g respectively. The value obtained at 4.8 ºC represented a reduction of 67 % compared with log $N_{\text{max}}$ estimated in mono-culture in fish fillets (5.68 log cfu/g).

The average pH values for fish fillets remained constant throughout the storage time (6.15 ± 0.02) and the gas concentration in the packaging at the end of storage was 31.2 % ± 0.85 and 29.0 % ± 0.19 for $O_2$ and $CO_2$, respectively.

The kinetic parameters, in the model, for both microorganisms were estimated by using the secondary models ($\lambda$ and $\mu_{\text{max}}$) derived from mono-culture experiments except for
log $N_{\text{max}}$ which was not temperature dependent and therefore, the average value was
used instead (i.e., in log scale, 7.92 and 8.74 log cfu/mL for *L. sakei* and *L. monocytogenes*, respectively). To consider the effect of food matrix on $\mu_{\text{max}}$ ($\text{h}^{-1}$), the
reduction of this parameter on the fresh fish product (4.8 ºC) in relation to that observed
in fish juice in mono-culture was estimated (5.0 ºC), which corresponded to 0.68. Thus,
for simulating growth, the specific growth rate for *L. monocytogenes* was adjusted
applying the above reduction rate in the Lotka Volterra model. Due to the difficulty to
set an equation describing the temperature effect on the competition factor, this was
fixed to the average of the values obtained at the different temperatures at the ratio 1:2,
which corresponded to 1.54. It was deemed that the value was representative for the
assayed temperatures, considering that most temperatures in challenge tests were in the
range 4-12 ºC, where the competition factor was similar. The same reduction rate and
competition factor were used for the experiments at isothermal conditions (4.8 and 8.2
ºC) as well as for the two dynamic time-temperature profiles.

Table 6 shows RMSE and ASZ values for the growth interaction of *L. sakei* CTC494
and *L. monocytogenes* CTC1034 on fish fillets predicted by Lotka Volterra model. The
RMSE values for experiments under isothermal conditions varied between 0.378-0.555
and 0.452-0.593 for *L. sakei* CTC494 and *L. monocytogenes* CTC1034, respectively.
The visual inspection of the simulated line also confirmed that good performance of
models, demonstrating that the model was able to simulate the observed slight *Listeria*
increase and subsequent decline, though at 8.2 ºC, observations showed a more
prominent decline than the one predicted by the model simulation (i.e. fail-safe
prediction). Furthermore, values for ASZ considering as criterion ± 0.5 log.units showed
that models can mostly accounted for the counts recorded during the interaction experiments, with values of 79 % (Table 6).

For non-isothermal temperature conditions, RMSE values ranged from 0.645-0.894 and 0.309-0.615 for *L. sakei* and *L. monocytogenes*, respectively. Lotka Volterra model showed closer predictions to experimental data in fish fillets for *L. monocytogenes* under profile 1. The percentages for the ASZ corresponded to 92 % (11/12) and 77 % (10/13) for profile 1 and 2, respectively. For *L. sakei*, ASZ values varied between 75 (9/12) and 77 % (10/13) for both profiles. Lotka Volterra model overestimated the exponential phase of *L. sakei* CTC494, while for *L. monocytogenes* the same was observed only for profile 2. The overestimation in profiles for *L. monocytogenes* could be considered as a “fail-safe” prediction since growth was predicted when no-growth was actually observed (i.e. profile 2).

**4. Discussion**

**4.1. L. monocytogenes growth in mono-culture**

For *L. monocytogenes* in mono-culture at 5° C, $\mu_{\text{max}}$ values obtained in our study were 16 % higher than those found by Verheyen et al. (2018) for in fish-protein based emulsions at 4 °C used as food model system for fish. On the contrary, the $\mu_{\text{max}}$ observed by Bolívar et al. (2018) in fish juice within the interval 5-11 °C were higher (30-57%) than those found in our study in the range 4-12 °C. Differences in growth rates could be mainly attributed to strain variability and experimental conditions. By the contrary, the predictions provided by Combase Predictor ([https://www.combase.cc/index.php/en/](https://www.combase.cc/index.php/en/)) considering the same physico-chemical characteristics as those obtained for fish juice (pH = 6.66; $a_w = 0.997$) were similar in all temperatures studied. Furthermore, the obtained $\mu_{\text{max}}$ for *L. monocytogenes* in our fish...
juice were in the range of values reported for other fish matrices (i.e. 0.0329-0.2075 at 4-12 °C) such as smoked salmon, raw tuna, vacuum-packed rainbow trout fillets and sea bream fillets under MAP conditions (Faber, 1991; Hisar et al., 2005; Hwang, 2007; Liu et al., 2016; Provincial et al., 2013).

4.2. Growth interaction of L. sakei CTC494 and L. monocytogenes CTC1034

In general, observations in our study showed that the suppression of Listeria growth occurred when the dominant population, i.e. L. sakei CTC494 reached their $N_{max}$. This result would signal a potential Jameson effect between populations. Several studies have considered the Jameson effect in the simultaneous growth of microorganisms and L. monocytogenes on fish products (Beaufort et al., 2007; Giménez and Dalgaard, 2004; Koseki et al., 2011; Mejlholm and Dalgaard, 2007).

According to results, the inhibitory effect was influenced by the inoculation ratio and temperature, which has been also reported in other works (Quinto et al., 2016; Yamazaki et al., 2003). Differences in inoculum level is key to determine the dominant microorganism in the microbial interaction and thus, the level of inhibition between microbial populations (Mellefont et al., 2008). Despite this fact, we observed that L. sakei CTC494 exerted a slight inhibition on $N_{max}$ of L. monocytogenes even when both microorganisms were inoculated the same level (ratio 1:1). This inhibition at equal inoculum level could be associated with production of bacteriocin since L. sakei CTC494 produces sakacin K (Hugas et al., 1995; De Vuyst and Leroy, 2007; Leroy et al., 2005; Ravyts et al., 2008) and the influence of other metabolites such as organic acids was discarded as potential inhibitors because of no relevant changes in pH were detected during growth experiments in fish juice and fish samples.
In summary, the interaction between *L. sakei* CTC494 and *L. monocytogenes* presented in our study could be understood by a combination of two mechanisms: i) a non-specific interaction involving the Jameson effect on the inhibition of *L. monocytogenes*, occurring when *L. sakei* CTC494 is present at an initial concentration higher than *L. monocytogenes* together with the fact that the bioprotective strain grows faster than the pathogen (Mellefont et al., 2008; Jameson, 1962) and ii) specific interaction caused by modification of the medium where both microorganisms coexist, resulting in an specific antagonistic effect on the growth of *L. monocytogenes* due to bacteriocin production (i.e. sakacin K) by the bioprotective strain (Aguilar and Klotz, 2010; Vescovo et al., 2006). However, the production of bacteriocin was not quantified in our study, thus no conclusion can be drawn about which interaction phenomenon was more relevant. Nor could mechanistic models be applied due to the lack of biological insight into the metabolic and genetic phenomena arising from the simultaneous growth of two microbial populations.

### 4.3. Lotka Volterra’s competition factor

The competition factors for *L. monocytogenes* (*F*<sub*LSTM*</sub>) in fish juice were slightly temperature dependent for all ratios (Table 5). The largest increase in the competition factor took place at low temperatures for ratios 1:2 and 1:3 (i.e. 2.2-5 °C) while for ratio 1:1, higher temperatures (8-12 °C) were responsible for a higher rise of this factor. No mathematical expression could be derived from data because of the limited number of observations, reduced temperature range and the lack of a clear pattern in data. Møller et al. (2013) estimated the competition factors for natural microbiota on growth of *Salmonella* spp. in fresh pork using the Lotka Volterra model and expanded Jameson effect model and found dependency on range of storage temperature assayed. By the
contrary, Mejholm and Dalgaard (2015) using the model proposed by Giménez and Dalgaard (2004) did not find that the competition factor was temperature dependent. Furthermore, the traditional Jameson effect model or its modification suggested by Le Marc et al. (2009) have been used to predict growth of microorganisms in food at different storage temperature (Giménez and Dalgaard, 2004; Le Marc et al., 2009; Mejholm and Dalgaard, 2007; Vermeulen et al., 2011). In those studies, however, the effect of microbial interaction on growth patterns was independent of the studied storage temperatures. The divergence between studies to correlate interaction factors with temperature can be related to the different conditions used in experiments (i.e. type of microorganism, food matrix and inoculum concentration).

Competition factors, in our study, were also under the influence of the inoculation ratio. Thus, the lowest values were obtained for ratios 1:1 and 1:2 (Table 5). Baka et al. (2014) estimated low competition factors for the interaction between *Leuconostoc carnosum* and *L. monocytogenes* in vacuum packed Frankfurter sausages stored at 4 °C for the ratio 1:1. At intermediate temperatures (8.1-12.1 °C), the competition factors decreased with the initial increase of concentration of *Leuconostoc carnosum*. Fujikawa et al. (2014) found that the values for the competition factors did not vary with the combinations of the initial populations of *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* spp. at 28 °C.

According to Baka et al. (2014), differences in values of the competition factor can be attributed to the combination of different variables, such as temperature, nutrient depletion, pH, bacteriocin production, organic acid, MAP conditions, etc., which can be considered as part of the hurdle concept (Leistner, 1995). When these variables are identified, the Lotka Volterra model can be modified for more realistic microbial
interaction descriptions, for instance, the effect of environmental conditions (i.e. temperature), the influence of inhibitory substances on lag phase duration of pathogenic organisms or whether bacteriocin production is dependent quorum sensing (Dens et al., 1999, Powell et al., 2004).

4.4. Simulating growth inhibition and bioprotective activity of L. sakei CTC494 on L. monocytogenes CTC1034 on fish fillets

A challenge test on gilthead sea bream fillets under MAP inoculated with L. monocytogenes and L. sakei CTC494 in co-culture at ratio 1:2 was carried out under isothermal and non-isothermal conditions. The Lotka Volterra model slightly overestimated the experimental observations of L. sakei in the exponential phase for the profiles 1 and 2. These discrepancies can be partly explained by the fact that the performance of a dynamic model depends on the performance of the primary and secondary models, and the sudden temperature changes can cause an intermediate lag time that cannot be predicted by the models (Longhi et al., 2013). Nevertheless, this fact did not affect predictions for Listeria growth, providing a reliable estimate for this pathogen for two dynamic conditions, according to the ASZ approach.

The control of pathogenic bacteria using LAB as bioprotective cultures in fish products is widely reported in literature (Bernardi et al., 2011; Chowdhury et al., 2012; Ghanbari et al., 2013; Hisar et al., 2005; Matamoros et al., 2009; Nath et al., 2014; Tahiri et al., 2009; Tomé et al., 2008; Weiss and Hammes, 2006), thus showing that live microbiological cultures can be a more effective alternative to the use of bacteriocins (Pilet and Leroy, 2011), which in addition are not permitted by most of the food additive regulations. However, the selection of candidates as bioprotective cultures to improve food quality and extend shelf-life has been attributed to the capacity not to
produce undesirable organoleptic changes in foods. In this sense, *L. sakei* CTC494 is reported in literature as a starter culture providing good organoleptic and sensory properties in fermented meat products and as bioprotective (not spoiling) culture in cooked ham (Aymerich et al., 2002; Bover-Cid et al., 2001; Hugas et al., 1995; Hugas et al., 1998; Hugas et al., 2002). Our study proposed the extension of the use the bacteriocinogenic *L. sakei* CTC494 in raw fish and other minimally processed fish products demonstrating that its availability to grow in a different food matrix and its application as a suitable approach for controlling *L. monocytogenes* growth in packaged sea bream fillets stored under isothermal and non-isothermal conditions including moderate abuse.

5. Conclusion

Results demonstrated that the use of the bacteriocinogenic strain *L. sakei* CTC494, as bioprotective culture is a suitable strategy for controlling *L. monocytogenes* growth in minimally processed fresh fish products (i.e. filleted gilthead sea bream) under refrigerated storage. Furthermore, the modelling approach, developed herein, based on a step-wise scheme from mono-culture experiments in fish juice under isothermal conditions to experiments performed in co-culture in actual fish product under dynamic temperature profiles was proved to be effective to derive reliable microbial interaction models. These mathematical models could be used as a predictive tool to simulate the simultaneous behaviour of bioprotective lactobacillus strain and *L. monocytogenes*. Thus, these tools can support the design and optimization of bioprotective culture based strategies against *L. monocytogenes* in minimally processed fish products.

Supplementary data to this article can be found online at.
Acknowledgements

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Declarations of interest

The authors declare that there is no conflict of interest in the publication of this paper.

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**Supplementary Table S1.** Quality Index Method (QIM) scheme applied to the sensory analysis of filleted sea bream under modified atmosphere packaging adapted from Lougoivois et al. (2003) and Campus et al. (2011).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Attributes</th>
<th>Demerit points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Skin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bright, shining, iridescent</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Less bright, some loss of iridescence</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Pale, dull</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Slime/Mucus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clear-transparent</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Slightly cloudy/cloudy</td>
<td>1</td>
</tr>
<tr>
<td>Flesh</td>
<td>Colour</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fresh, translucent</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Waxy, milky</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Dull, slightly discoloured, yellowish</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Stiffness</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Firm</td>
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</tr>
<tr>
<td></td>
<td>Some softening</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Soft</td>
<td>2</td>
</tr>
<tr>
<td>Odour</td>
<td>Odour</td>
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</tr>
<tr>
<td></td>
<td>Neutral</td>
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</tr>
<tr>
<td></td>
<td>Slight off-odours</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Spoiled</td>
<td>3</td>
</tr>
<tr>
<td>Quality Index (QI, as the sum of assigned demerit points)</td>
<td>0-10</td>
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</tbody>
</table>
### Supplementary Table S2. Goodness-of-fit indexes for the fit of the Baranyi and Roberts model to the growth data of *L. sakei* CTC494 and *L. monocytogenes* CTC1034 in mono-culture in sterile fish juice of sea bream and sea bream fillets under modified atmosphere packaging at different temperatures.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Temp. (°C)</th>
<th>n (Ls)</th>
<th>n (Lm)</th>
<th><em>Lactobacillus sakei</em> CTC494</th>
<th><em>Listeria monocytogenes</em> CTC1034</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RMSE</td>
<td>R²</td>
</tr>
<tr>
<td><strong>Fish juice</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.2</td>
<td>12</td>
<td>19</td>
<td>0.061</td>
<td>0.9991&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>18</td>
<td>23</td>
<td>0.107</td>
<td>0.9972</td>
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<tr>
<td></td>
<td>8.1</td>
<td>14</td>
<td>14</td>
<td>0.120</td>
<td>0.9968</td>
</tr>
<tr>
<td></td>
<td>12.1</td>
<td>14</td>
<td>17</td>
<td>0.126</td>
<td>0.9965</td>
</tr>
<tr>
<td><strong>Fresh fish fillets</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.8</td>
<td>7</td>
<td>11</td>
<td>0.191</td>
<td>0.9930</td>
</tr>
</tbody>
</table>

<sup>a</sup>Experiments in sterile fish juice of gilthead sea bream inoculated with ca. 10<sup>2</sup> cfu/mL of *L. sakei* or *L. monocytogenes*.

<sup>b</sup>Experiments on gilthead sea bream fillets under modified atmosphere packing inoculated with ca. 10<sup>2</sup> cfu/g of *L. sakei* or *L. monocytogenes*.

<sup>c</sup>n (Ls), number of data (sampling points) for *L. sakei* CTC494.

<sup>d</sup>n (Lm), number of data (sampling points) for *L. monocytogenes* CTC1034.

<sup>e</sup>RMSE, Root mean square error.

<sup>f</sup>R², Coefficient of determination.
Supplementary Table S3. Goodness-of-fit indexes for the Baranyi and Roberts model without interaction fitted to co-culture growth data of *L. sakei* CTC494 and *L. monocytogenes* CTC1034 in sterile fish juice of sea bream and sea bream fillets under modified atmosphere packaging at different temperature.

<table>
<thead>
<tr>
<th>Ratio&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Temp. (°C)</th>
<th>n (Ls)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>n (Lm)&lt;sup&gt;c&lt;/sup&gt;</th>
<th><strong>Lactobacillus sakei</strong> CTC494</th>
<th><strong>Listeria monocytogenes</strong> CTC1034</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RMSE&lt;sup&gt;d&lt;/sup&gt;</td>
<td>R&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>1:1</td>
<td>2.2</td>
<td>17</td>
<td>17</td>
<td>0.100</td>
<td>0.9979&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>1:2</td>
<td>1:3</td>
<td>14</td>
<td>14</td>
<td>0.081</td>
<td>0.9963</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11</td>
<td>11</td>
<td>0.071</td>
<td>0.9818</td>
</tr>
<tr>
<td>1:1</td>
<td>5.0</td>
<td>14</td>
<td>14</td>
<td>0.113</td>
<td>0.9968</td>
</tr>
<tr>
<td>1:2</td>
<td>1:3</td>
<td>13</td>
<td>7</td>
<td>0.117</td>
<td>0.9924</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>10</td>
<td>0.023</td>
<td>0.9969</td>
</tr>
<tr>
<td>1:1</td>
<td>8.1</td>
<td>14</td>
<td>14</td>
<td>0.135</td>
<td>0.9965</td>
</tr>
<tr>
<td>1:2</td>
<td>1:3</td>
<td>11</td>
<td>7</td>
<td>0.060</td>
<td>0.9986</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11</td>
<td>11</td>
<td>0.092</td>
<td>0.9800</td>
</tr>
<tr>
<td>1:1</td>
<td>12.1</td>
<td>16</td>
<td>11</td>
<td>0.128</td>
<td>0.9965</td>
</tr>
<tr>
<td>1:2</td>
<td>1:3</td>
<td>11</td>
<td>7</td>
<td>0.081</td>
<td>0.9970</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11</td>
<td>11</td>
<td>0.100</td>
<td>0.9761</td>
</tr>
</tbody>
</table>

<sup>a</sup>Ratio of inoculation of *L. monocytogenes* CTC1034 and *L. sakei* CTC494 in sterile fish juice of gilthead sea bream where the ratios 1:1, 1:2 or 1:3 represent the initial concentrations of 2 log cfu/mL for the *L. monocytogenes* strain and 2, 4, 6 log cfu/mL for the *L. sakei* strain, respectively.

<sup>b</sup>n (Ls), number of data (sampling points) for *L. sakei* CTC494.

<sup>c</sup>n (Lm), number of data (sampling points) for *L. monocytogenes* CTC1034.

<sup>d</sup>RMSE, Root mean square error.

<sup>e</sup>R<sup>2</sup>, Coefficient of determination.

<sup>f</sup>NF, no fit as no growth was observed.
Table 1. Estimated lag time ($\lambda$), maximum specific growth rate ($\mu_{\text{max}}$) and $N_{\text{max}}$ (maximum population density) and associated standard errors for *L. sakei* CTC494 and *L. monocytogenes* CTC1034 in mono-culture obtained from the Baranyi and Roberts model in sterile fish juice of sea bream and sea bream fillets under modified atmosphere packaging.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Temp. (°C)</th>
<th>$\log N_0$ (Ls) (log cfu/mL or g)</th>
<th>$\lambda$ (h)</th>
<th>$\mu_{\text{max}}$ (h$^{-1}$)</th>
<th>$\log N_{\text{max}}$ (log cfu/mL or g)</th>
<th>$\log N_0$ (Lm) (log cfu/mL or g)</th>
<th>$\lambda$ (h)</th>
<th>$\mu_{\text{max}}$ (h$^{-1}$)</th>
<th>$\log N_{\text{max}}$ (log cfu/mL or g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish juice$^a$</td>
<td>2.2</td>
<td>2.36</td>
<td>92.4 ± 7.55</td>
<td>0.0351 ± 0.0004</td>
<td>7.70 ± 0.03</td>
<td>2.59</td>
<td>166.7 ± 23.20</td>
<td>0.0226 ± 0.0004</td>
<td>8.92 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>2.04</td>
<td>43.1 ± 6.72</td>
<td>0.0697 ± 0.0005</td>
<td>7.85 ± 0.05</td>
<td>1.53</td>
<td>36.1 ± 7.13</td>
<td>0.0477 ± 0.0005</td>
<td>8.65 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>8.1</td>
<td>2.67</td>
<td>18.7 ± 5.04</td>
<td>0.1273 ± 0.0039</td>
<td>7.94 ± 0.05</td>
<td>2.29</td>
<td>15.1 ± 6.26</td>
<td>0.0892 ± 0.0019</td>
<td>8.68 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>12.1</td>
<td>2.48</td>
<td>5.3 ± 2.68</td>
<td>0.2140 ± 0.0052</td>
<td>8.17 ± 0.07</td>
<td>2.39</td>
<td>2.0 ± 2.01</td>
<td>0.1685 ± 0.0020</td>
<td>8.70 ± 0.06</td>
</tr>
<tr>
<td>Fresh fish fillets$^b$</td>
<td>4.8</td>
<td>1.49</td>
<td>33.8 ± 11.39</td>
<td>0.0806 ± 0.0036</td>
<td>7.08 ± 0.13</td>
<td>2.71</td>
<td>56.1 ± 35.23</td>
<td>0.0154 ± 0.0006</td>
<td>5.68 ± 0.13</td>
</tr>
</tbody>
</table>

$^a$ Experiments in sterile fish juice of gilthead sea bream inoculated with ca. $10^2$ cfu/mL of *L. sakei* or *L. monocytogenes*.

$^b$ Experiments on gilthead sea bream fillets under modified atmosphere packing inoculated with ca. $10^2$ cfu/g of *L. sakei* or *L. monocytogenes*. 
Table 2. Coefficients of the square-root model describing the effect of temperature on lag time ($\lambda$) and maximum specific growth rate ($\mu_{\text{max}}$) of *L. sakei* CTC494 and *L. monocytogenes* CTC1034 in sterile fish juice of sea bream.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Microorganisms</th>
<th>$b$</th>
<th>$T_{\text{min}}$ (°C)</th>
<th>RMSE$^a$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda$</td>
<td><em>L. sakei</em> CTC494</td>
<td>-0.7269</td>
<td>14.69</td>
<td>7.365</td>
<td>0.9695</td>
</tr>
<tr>
<td></td>
<td><em>L. monocytogenes</em> CTC1034</td>
<td>-1.0868</td>
<td>12.42</td>
<td>30.332</td>
<td>0.8737</td>
</tr>
<tr>
<td>$\mu_{\text{max}}$</td>
<td><em>L. sakei</em> CTC494</td>
<td>0.0280</td>
<td>-4.50</td>
<td>0.086</td>
<td>0.9994</td>
</tr>
<tr>
<td></td>
<td><em>L. monocytogenes</em> CTC1034</td>
<td>0.0263</td>
<td>-3.40</td>
<td>0.064</td>
<td>0.9990</td>
</tr>
</tbody>
</table>

$^a$RMSE, Root mean square error.

$^b$R$^2$, Coefficient of determination.
Table 3. Estimated lag time ($\lambda$), maximum specific growth rate ($\mu_{\text{max}}$), $N_{\text{max}}$ (maximum population density) and associated standard error from the Baranyi and Roberts model without interaction fitted to the growth of *L. sakei* CTC494 and *L. monocytogenes* CTC1034 in co-culture in sterile fish juice of sea bream.

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Temp. (°C)</th>
<th>$\log N_0$ (Ls) log cfu/mL</th>
<th>$\lambda$ (h)</th>
<th>$\mu_{\text{max}}$ (h$^{-1}$)</th>
<th>$\log N_{\text{max}}$ (log cfu/mL)</th>
<th>$\log N_0$ (Lm) log cfu/mL</th>
<th>$\lambda$ (h)</th>
<th>$\mu_{\text{max}}$ (h$^{-1}$)</th>
<th>$\log N_{\text{max}}$ (log cfu/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1</td>
<td>2.2</td>
<td>2.37</td>
<td>53.3 ± 11.72</td>
<td>0.0350 ± 0.0007</td>
<td>7.74 ± 0.05</td>
<td>2.52</td>
<td>75.4 ± 16.32</td>
<td>0.0223 ± 0.0006</td>
<td>5.77 ± 0.04</td>
</tr>
<tr>
<td>1:2</td>
<td>5.0</td>
<td>4.58</td>
<td>59.5 ± 8.08</td>
<td>0.0355 ± 0.0097</td>
<td>7.63 ± 0.04</td>
<td>2.60</td>
<td>106.1 ± 6.45</td>
<td>0.0268 ± 0.0008</td>
<td>4.28 ± 0.01</td>
</tr>
<tr>
<td>1:3</td>
<td>6.62</td>
<td>6.77</td>
<td>39.4 ± 14.39</td>
<td>0.0284 ± 0.0027</td>
<td>7.99 ± 0.03</td>
<td>2.57</td>
<td>NG</td>
<td>5.94 ± 0.03</td>
<td>4.22 ± 7.35</td>
</tr>
<tr>
<td>1:1</td>
<td>5.0</td>
<td>2.84</td>
<td>30.0 ± 4.72</td>
<td>0.0675 ± 0.0012</td>
<td>7.94 ± 0.07</td>
<td>2.42</td>
<td>36.9 ± 6.87</td>
<td>0.0490 ± 0.0012</td>
<td>5.94 ± 0.03</td>
</tr>
<tr>
<td>1:2</td>
<td>4.59</td>
<td>4.59</td>
<td>23.0 ± 7.52</td>
<td>0.0625 ± 0.0026</td>
<td>7.86 ± 0.05</td>
<td>2.49</td>
<td>14.5 ± 18.80</td>
<td>0.0334 ± 0.0053</td>
<td>4.22 ± 7.35</td>
</tr>
<tr>
<td>1:3</td>
<td>6.62</td>
<td>6.62</td>
<td>11.8 ± 3.13</td>
<td>0.0294 ± 0.0007</td>
<td>7.68 ± 0.01</td>
<td>2.46*</td>
<td>NG</td>
<td>6.07 ± 0.18</td>
<td>4.03 ± 11</td>
</tr>
<tr>
<td>1:1</td>
<td>5.0</td>
<td>2.42</td>
<td>20.3 ± 3.50</td>
<td>0.1373 ± 0.0029</td>
<td>8.11 ± 0.06</td>
<td>2.27</td>
<td>12.3 ± 19.73</td>
<td>0.1085 ± 0.0131</td>
<td>6.07 ± 0.18</td>
</tr>
<tr>
<td>1:2</td>
<td>8.1</td>
<td>4.34</td>
<td>15.2 ± 2.15</td>
<td>0.1266 ± 0.0023</td>
<td>8.14 ± 0.03</td>
<td>1.92</td>
<td>0.0 ± 0.00</td>
<td>0.0977 ± 0.0058</td>
<td>4.03 ± 11</td>
</tr>
<tr>
<td>1:3</td>
<td>6.37</td>
<td>6.37</td>
<td>0.0 ± 0.00</td>
<td>0.0703 ± 0.0027</td>
<td>7.96 ± 0.03</td>
<td>2.24*</td>
<td>NG</td>
<td>6.81 ± 0.08</td>
<td>4.86 ± 12</td>
</tr>
<tr>
<td>1:1</td>
<td>5.0</td>
<td>2.49</td>
<td>6.4 ± 1.62</td>
<td>0.2292 ± 0.0035</td>
<td>8.17 ± 0.06</td>
<td>2.33</td>
<td>1.3 ± 1.86</td>
<td>0.1959 ± 0.0035</td>
<td>6.81 ± 0.08</td>
</tr>
<tr>
<td>1:2</td>
<td>12.1</td>
<td>4.40</td>
<td>5.1 ± 1.16</td>
<td>0.2273 ± 0.0046</td>
<td>8.20 ± 0.03</td>
<td>2.47</td>
<td>0.0 ± 0.00</td>
<td>0.1733 ± 0.0065</td>
<td>4.86 ± 12</td>
</tr>
<tr>
<td>1:3</td>
<td>6.37</td>
<td>6.37</td>
<td>0.0 ± 0.00</td>
<td>0.1716 ± 0.0073</td>
<td>8.06 ± 0.03</td>
<td>2.47*</td>
<td>NG</td>
<td>NG</td>
<td>4.86 ± 12</td>
</tr>
</tbody>
</table>

*a* Ratio of inoculation of *L. monocytogenes* CTC1034 and *L. sakei* CTC494 in fish juice of sea bream where the ratios 1:1, 1:2 or 1:3 represent the initial concentrations of 2 log cfu/mL for the *L. monocytogenes* strain and 2, 4, 6 log cfu/mL for the *L. sakei* strain, respectively.

*b* NG, no growth.

* Observed initial concentration of *L. monocytogenes*. 


Table 4. Quality Index values obtained from the sensory analysis of sea bream fillets packaged under modified atmosphere and stored under refrigerated conditions (5 °C, 8 days) for samples inoculated at a ratio 1:2 (*Listeria monocytogenes: Lactobacillus sakei*) (i.e., 2 log cfu/g and 4 log cfu/g, respectively) and control fillets (non-inoculated).

<table>
<thead>
<tr>
<th>Storage time (days)</th>
<th>Quality Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inoculated fillets</td>
</tr>
<tr>
<td>0</td>
<td>0.3 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>0.6 ± 1.3</td>
</tr>
<tr>
<td>6</td>
<td>2.0 ± 1.7</td>
</tr>
<tr>
<td>8</td>
<td>6.3 ± 0.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Storage under modified atmosphere packaging at 5 °C.

<sup>b</sup> Mean ± standard deviation (n = 5 panelists).
Table 5. Estimated maximum critical concentration ($N_{crit}$) of the modified Jameson effect model and competition factors ($F_{LmLs}$ and $F_{LsLm}$) of the Lotka Volterra model and goodness-of-fit index (RMSE) for *L. sakei* CTC494 and *L. monocytogenes* CTC1034 in co-culture in fish juice of sea bream.

<table>
<thead>
<tr>
<th>Ratios a</th>
<th>Temp. (°C)</th>
<th>n b</th>
<th>Jameson model</th>
<th>Modified Jameson model</th>
<th>Lotka Volterra model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Lactobacillus sakei</em> CTC494</td>
<td><em>Listeria monocytogenes</em> CTC1034</td>
<td><em>Lactobacillus sakei</em> CTC494</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RMSE c</td>
<td>RMSE</td>
<td>RMSE</td>
</tr>
<tr>
<td>1:1</td>
<td>17</td>
<td>0.314</td>
<td>0.423</td>
<td>0.349</td>
<td>1.00*10^6</td>
</tr>
<tr>
<td>1:2</td>
<td>2.2</td>
<td>14</td>
<td>0.293</td>
<td>0.189</td>
<td>0.335</td>
</tr>
<tr>
<td>1:3</td>
<td>11</td>
<td>0.224</td>
<td>1.214</td>
<td>0.350</td>
<td>1.00*10^6</td>
</tr>
<tr>
<td>1:1</td>
<td>14</td>
<td>0.370</td>
<td>0.387</td>
<td>0.371</td>
<td>1.00*10^6</td>
</tr>
<tr>
<td>1:2</td>
<td>5.0</td>
<td>13</td>
<td>0.279</td>
<td>1.058</td>
<td>0.322</td>
</tr>
<tr>
<td>1:3</td>
<td>10</td>
<td>0.139</td>
<td>1.240</td>
<td>0.139</td>
<td>1.00*10^6</td>
</tr>
<tr>
<td>1:1</td>
<td>14</td>
<td>0.216</td>
<td>0.666</td>
<td>0.217</td>
<td>1.00*10^6</td>
</tr>
<tr>
<td>1:2</td>
<td>8.1</td>
<td>11</td>
<td>0.300</td>
<td>0.930</td>
<td>0.300</td>
</tr>
<tr>
<td>1:3</td>
<td>11</td>
<td>0.190</td>
<td>1.796</td>
<td>0.190</td>
<td>1.00*10^6</td>
</tr>
<tr>
<td>1:1</td>
<td>16</td>
<td>0.213</td>
<td>1.138</td>
<td>0.210</td>
<td>9.99*10^7</td>
</tr>
<tr>
<td>1:2</td>
<td>12.1</td>
<td>14</td>
<td>0.108</td>
<td>1.424</td>
<td>0.108</td>
</tr>
<tr>
<td>1:3</td>
<td>11</td>
<td>0.105</td>
<td>2.050</td>
<td>0.105</td>
<td>1.00*10^6</td>
</tr>
</tbody>
</table>

a Ratio of inoculation of *L. monocytogenes* CTC1034 and *L. sakei* CTC494 in sterile fish juice where the ratios 1:1, 1:2 or 1:3 represent the initial concentrations of $2 \log$ cfu/mL for the *L. monocytogenes* strain and 2, 4, 6 log cfu/mL for the *L. sakei* strain, respectively.

b n, number of data (sampling points) for *L. sakei* CTC494 and *L. monocytogenes*.
5. RMSE, Root mean square error.

6. $L_m$, maximum critical concentration for *L. monocytogenes* CTC1034 obtained from the Jameson’s modified model.

7. $L_s$, maximum critical concentration for *L. sakei* CTC494 obtained from the Jameson’s modified model.

8. $F_{LM}$, competition factor of *L. monocytogenes* CTC1034 in *L. sakei* CTC494 obtained from the Lotka Volterra model.

9. $F_{SL}$, competition factor of *L. sakei* CTC494 in *L. monocytogenes* CTC1034 obtained from the Lotka Volterra model.
Table 6. Predictive performance of the Lotka Volterra model when applied to simulate the simultaneous growth of *L. sakei* CTC494 and *L. monocytogenes* CTC1034 in sea bream fillets under modified atmosphere packaging stored under isothermal and non-isothermal conditions.

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>n(^a)</th>
<th><em>Lactobacillus sakei</em></th>
<th><em>Listeria monocytogenes</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(N_0) (LAB) cfu/g</td>
<td>RMSE(^b) ASZ(^c)</td>
</tr>
<tr>
<td>4.8</td>
<td>14</td>
<td>3.36</td>
<td>0.555 79 %</td>
</tr>
<tr>
<td>8.2</td>
<td>14</td>
<td>3.55</td>
<td>0.378 79 %</td>
</tr>
<tr>
<td>Profile 1</td>
<td>12</td>
<td>3.71</td>
<td>0.894 75 %</td>
</tr>
<tr>
<td>(4-8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Profile 2</td>
<td>13</td>
<td>3.96</td>
<td>0.645 77 %</td>
</tr>
<tr>
<td>(2.5-12)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) n, number of data (sampling points) for *L. sakei* CTC494 and *L. monocytogenes* CTC1034.

\(^b\) RMSE, Root mean square error

\(^c\) ASZ, acceptable simulation zone defined as ± 0.5 log-units from the simulated log cfu/g values (Møller et al., 2013).
Figure 1. A schematic overview of the modelling approach used in this study. Solid lines represent the experiments carried out for data generation, while dotted and dashed lines stand for the model building process and interaction model simulation, respectively.

Figure 2. Reduction ratio ($\alpha$) of the parameters (a) lag time ($\lambda$) and (b) $N_{\text{max}}$ for *Listeria monocytogenes* CTC1034 (black bars) and *Lactobacillus sakei* CTC494 (grey bars) in co-culture on sterile juice fish of sea bream at different storage temperatures with three inoculation ratios of *L. monocytogenes:* *L. sakei*. The negative bars represent an increase in co-culture for the specific parameter. No growth of *L. monocytogenes* was observed at the ratio 1:3 (NG).

Figure 3. Experimental observed data and fitted Jameson (dotted line), modified Jameson (dashed line) and Lotka Volterra (solid line) models for *Lactobacillus sakei* CTC494 (○) and *Listeria monocytogenes* CTC1034 (●) in sterile fish juice of sea bream stored at (a, b, c) 2.2, (d, e, f) 5.0, (g, h, i) 8.1 and (j, k, l) 12.1 °C for the inoculation ratios of *L. monocytogenes:* *L. sakei*, 1:1, 1:2 and 1:3, respectively. The grey dotted line stands for the storage temperature recorded.

Figure 4. Experimental observed data (mean and standard deviation of 3 replicates) and simulations provided by the predictive model based on the Lotka Volterra equation for *Lactobacillus sakei* CTC494 (○) and *Listeria monocytogenes* CTC1034 (●) on sea bream fillets under modified atmosphere packaging at isothermal conditions: (a) 4.8 °C, (b) 8.2 °C; and dynamic temperature conditions (c) profile 1 (4-8 °C) and (d) profile 2 (2.5-12.0 °C). Dashed and solid line represent the simulations for *L. sakei* and *L. monocytogenes* strains, respectively. Dotted lines show the acceptable simulation zone (ASZ) used to compare observations versus predictions of the interaction between *L. sakei* CTC494 and *L. monocytogenes* CTC1034. Grey dashed line stands for the storage temperature recorded.
Figure 1

Lag phase duration: $\lambda$, maximum specific growth rate: $\mu_{\text{max}}$, and maximum population density: $N_{\text{max}}$
Figure 2

(a) Conditions

(b) Conditions
Figure 3
Figure 4

(a) Log (cfu/g) vs. Time (days) at different temperatures.

(b) Log (cfu/g) vs. Time (days) at different temperatures.

(c) Log (cfu/g) vs. Time (days) at different temperatures.

(d) Log (cfu/g) vs. Time (days) at different temperatures.
Supplementary Figure S1. Growth curves of *Lactobacillus sakei* CTC494 (○) and *Listeria monocytogenes* CTC1034 (●) in mono-culture obtained in sterile fish juice of gilthead sea bream at (a) 2.2, (b) 5.0, (c) 8.1 and (d) 12.1 °C and (e) on sea bream fillets at 4.8 °C. Dashed line and solid line represent the fittings for the *L. sakei* and *L. monocytogenes* strains, respectively, obtained with the Baranyi and Roberts model.