



A comparison of dynamic tertiary and competition models for describing the fate of *Listeria monocytogenes* in Minas fresh cheese during refrigerated storage

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ABSTRACT

This study compares dynamic tertiary and competition models for *L. monocytogenes* growth as a function of intrinsic properties of a traditional Brazilian soft cheese and the inhibitory effect of lactic acid bacteria (LAB) during refrigerated storage. Cheeses were prepared from raw or pasteurized milk with or without the addition of selected LAB with known anti-listerial activity. Cheeses were analyzed for LAB and *L. monocytogenes* counts, pH and water activity (a_w) throughout cold storage. Two approaches were used to describe the effect of LAB on *L. monocytogenes*: a Huang-Cardinal model that considers the effect of pH and a_w variation in a dynamic kinetic analysis framework; and microbial competition models, including Lotka-Volterra and Jameson-effect variants, describing the simultaneous growth of *L. monocytogenes* and LAB. The Jameson-effect with γ and the Lotka-Volterra models produced models with statistically significant coefficients that characterized the inhibitory effect of selected LAB on *L. monocytogenes* in Minas fresh cheese. The Huang-Cardinal model [pH] outperformed both competition models. Taking a_w change into account did not improve the fit quality of the Huang-Cardinal [pH] model. These models for Minas soft cheese should be valuable for future microbial risk assessments for this culturally important traditional cheese.

1. Introduction

Listeria monocytogenes is an intracellular foodborne pathogen commonly associated to ready-to-eat products, and has been implicated as the causative agent of numerous disease outbreaks worldwide. According to Greig and Ravel (2009), 4093 foodborne outbreaks between 1988 and 2007, ~337 were related to dairy products, 6.6% of which were associated to *L. monocytogenes*. The organism has a fairly low prevalence in cheese (1.2% in European cheeses according to Martinez-Rios and Dalgaard, 2018) but can still cause outbreaks. Soft cheese contaminated with *L. monocytogenes* was responsible for an outbreak in Germany in 2006–2007 with 189 reported cases and 26 deaths (Koch et al., 2010), and in Portugal during 2009–2012 with 30 cases and a fatality rate of 36.7% (Magalhães et al., 2015). Fresh raw milk cheese in the USA was responsible for a listeriosis outbreak

involving 6 people (2 deaths) in 2016–2017 (CDC, 2017), while in Chile, a 2008 outbreak associated with Brie and Camembert cheeses caused 165 cases and 14 deaths (Montero et al., 2015). Listeriosis reporting is not currently compulsory in Brazil, and this lack of data may obscure the real magnitude of the problem (Barancelli et al., 2011). Brazilian *L. monocytogenes* cheese and dairy plants isolates do include the most virulent serotypes including 1/2a, 1/2b and 4b (Abrahão et al., 2008; Barancelli et al., 2014; Brito et al., 2008), which are the same types frequently involved in outbreaks of human listeriosis.

Minas “frescal” cheese is a soft white fresh cheese obtained by enzymatic coagulation of milk, which may be complemented by the addition of specific lactic acid bacteria (LAB) (BRAZIL, 1997). This soft cheese is very popular in Brazil and is typically characterized by 55–58% moisture, 17–19% fat, 1.4–1.6% salt and a pH range of 5.0–5.3 (Silva, 2005). *L. monocytogenes* have been shown to grow over a wide

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range of temperatures (1–45 °C) and pH values (4.0–9.5), and down to water activities (a_w) as low as 0.92 and salt concentration of up to 10% (Melo et al., 2015); which clearly include the attributes of Minas fresh cheese. The reported incidence of *L. monocytogenes* in this type of Brazilian cheese is quite variable (1.4–41.2%, Abrahão et al., 2008). *L. monocytogenes* can contaminate the product through the use of contaminated raw milk or by cross or post-process contamination of pasteurized products. While poor quality silage, inadequate animals housing, lack of hygiene during milking and the presence of mastitis in the herd are all associated with raw milk contamination by *L. monocytogenes* (Sanaa et al., 1993), the main sources at cheese processing plants are contaminated starter cultures, brines, floors, packaging materials, cheese vats, cheese clothes, curd cutting knives, cold rooms, production rooms and storage coolers (Kousta et al., 2010).

Several studies have analyzed *L. monocytogenes* behavior in Minas fresh cheese during processing and shelf-life (Malheiros et al., 2012; Naldini et al., 2009; Nascimento et al., 2008; Silva et al., 2014), but only Campagnollo et al. (2018) used primary predictive models to estimate the growth parameters of this pathogen. No previous work appears to have applied either competition models or dynamic tertiary models to describe the kinetics of *L. monocytogenes* in Brazilian cheeses. The development of such models is made more complex by the presence of indigenous or intentionally-added LAB, since these organisms produce antimicrobial compounds as lactic acid, hydrogen peroxide, diacetyl, reuterin and bacteriocins, which can inactivate or inhibit the growth of *L. monocytogenes* even during refrigerated storage (Guillier et al., 2008) as has been confirmed by many studies (Campagnollo et al., 2018; Guedes Neto et al., 2005; Ortolani et al., 2010; Ribeiro et al., 2014; Sip et al., 2012). Determination of growth parameters of *L. monocytogenes* and the development of models considering interactions with other microorganisms are important for assessment and management of the risk of listeriosis.

Only two studies have evaluated competition models to characterize the inhibitory effect of cheese natural microflora on the growth of *L. monocytogenes* to date. Guillier et al. (2008) investigated the inhibition mechanism of *L. monocytogenes* by the natural biofilm microflora on wooden shelves used in the ripening of a soft and smear cheese, while Østergaard et al. (2014) developed mathematical models for competition of mesophilic LAB from added cultures and *L. monocytogenes* during chilled storage of cottage cheese. Dynamic tertiary modeling (a combination of a primary model with a secondary model) is particularly suited to fermentation processes (Rosshaug et al., 2012; Schwartzman et al., 2011), but is as yet unexplored for use in *L. monocytogenes* modeling in fresh cheese. Such models have the capability to use dynamic data (i.e., microbial population data as driven by food intrinsic properties over time) for prediction.

This study compares dynamic tertiary and competition models for characterizing the suppression of *L. monocytogenes* growth, as affected either by the changing intrinsic properties of a traditional Minas soft cheese during refrigerated storage or by the inhibitory effect of LAB present. Brazilian Minas soft cheeses were prepared from raw or pasteurized milk with autochthonous (naturally present) LAB or addition of selected LAB with known anti-listerial activity.

2. Material and methods

2.1. LAB and *L. monocytogenes* strains and preparation of cell suspensions

Six LAB strains, identified as *Lactobacillus brevis*, *Lactobacillus plantarum* and *Enterococcus faecalis* (4 strains) previously isolated from artisanal Minas cheeses and with known anti-listerial activity (Campagnollo et al., 2018) were used as a LAB pool to be added during cheese production. Preparation of LAB strains was carried out as described by Campagnollo et al. (2018) using MRS broth (de Man, Rogosa and Sharpe – Acumedia, Neogen Corporation, Lansing/MI).

Two *L. monocytogenes* strains (*L. monocytogenes* strain 3968 -

serotype 1/2b and *L. monocytogenes* strain 3973 - serotype 4b, isolated from cheese and raw milk, respectively), kindly donated by Oswaldo Cruz Foundation (Rio de Janeiro/RJ/Brazil), were used in this study. Each strain of *L. monocytogenes* was cultured separately in TSBYE (Tryptic soy broth – Merck, Darmstadt, Germany, added of 0.6% Yeast Extract – Acumedia, Neogen Corporation, Lansing/MI) and cell suspensions (10^8 CFU/mL) were prepared according to Sant'Ana et al. (2012).

2.2. Minas fresh cheese production

Four different treatments were performed in duplicate, on different days, and consisted of production of soft fresh Minas cheese using raw or pasteurized milk, with or without the addition of selected LAB. *L. monocytogenes* was deliberately added in each treatment. Naturally occurring microbiota (e.g. autochthonous LAB) were assumed to be present in all treatments. Pooled selected LAB were added at 10^6 – 10^7 CFU/mL of milk, while pool *L. monocytogenes* strains were added at 10^5 – 10^6 CFU/mL of milk to simulate a high level of contamination. Production of soft Minas cheese was carried out as described by Campagnollo et al. (2018). Briefly, ten liters of milk were heated to $34 \pm 1^\circ\text{C}$ and added with 5 mL of CaCl_2 (saturated solution), 9 mL of commercial rennet Estrella (85% bovine pepsin + 15% bovine chymosin, Chr. Hansen, Valinhos/SP/Brazil) and selected LAB and/or *L. monocytogenes* strains, depending upon the treatment. After 40 min coagulation, curd cutting, slight agitation and resting for 30 min, sodium chloride (2 g/L) was added and curd was allowed to rest for another 30 min. The whey was drained and the curd was placed into perforated sterile cylindrical shapes. Cheeses were kept at room temperature for 1 h for dripping, turned upside down, and left for an additional 1 h for final dripping. Unmolded cheeses were packed in plastic bags, following storage at $7 \pm 1^\circ\text{C}$ for 15 days.

2.3. Microbiological and physicochemical analysis during refrigerated shelf-life

Microbiological and physicochemical analysis were performed throughout the refrigerated storage period of soft Minas cheeses, more precisely on day 0 (immediately after production), days 1, 2, 3 and 4 (twice a day, early morning and late afternoon) and days 5, 7, 9, 12 and 15 (once a day). Microbiological analysis included LAB counting in MRS agar (Acumedia, Neogen Corporation, Lansing/MI) and *L. monocytogenes* counting in Listeria Selective agar (Oxford Formulation – Oxoid, Basingstoke, UK), performed according to Njongmeta et al. (2015) and Ryser and Donnelly (2015), respectively. Physicochemical analysis consisted of determination of temperature and pH, using a portable pH meter coupled with a knife electrode and a temperature sensor (AK103 pH meter, SC18 electrode, Akso Electronic Products Ltda., São Leopoldo/RS/Brazil), and water activity (a_w) using an Aqualab water activity meter (model 4TE, Decagon Devices Inc., São José dos Campos/SP/Brazil).

2.4. Modeling the inhibition of *L. monocytogenes* in cheese during refrigerated shelf-life

None of the experimental *L. monocytogenes* growth curves exhibited noticeable lag phase. Two types of models were contrasted: one that describes the retardation by considering the effect of acidification (changing pH) and a_w variation in a dynamic kinetic analysis framework (i.e., indirect approach); and another that describes the simultaneous growth of *L. monocytogenes* and autochthonous/selected LAB (i.e., direct approach).

2.4.1. Describing the growth of *L. monocytogenes* by dynamic tertiary models

The growth of *L. monocytogenes* is expected to slow down as the

cheese matrix becomes more acidic and drier. The kinetic parameters of *L. monocytogenes* in cheese were determined by dynamic kinetic analysis by taking into consideration the drop in pH and a_w during storage at constant temperature (7 °C). This was accomplished by simultaneously fitting a primary growth model in differential form with an explicit secondary model of the specific growth rate as a function of the cheese intrinsic properties. The Huang model (Huang, 2008) was chosen as the primary model characterizing the growth of *L. monocytogenes* in cheese during storage, whilst the cardinal parameter model was chosen for secondary modeling. The effect of pH was considered in the cardinal parameter model (Rosso et al., 1995); and subsequently the term for a_w was added (Østergaard et al., 2014). Thus, the following two dynamic tertiary models were fitted to the data,

$$\frac{dY}{dt} = \frac{\mu_{max}}{1 + e^{-\alpha(t-\lambda)}} (1 - e^{Y-Y_{max}})$$

$$\mu_{max} = \mu_{opt} \left\{ \frac{(pH - pH_{min})(pH - pH_{max})}{(pH - pH_{min})(pH - pH_{max}) - (pH - pH_{opt})^2} \right\}$$

$$\frac{dY}{dt} = \frac{\mu_{max}}{1 + e^{-\alpha(t-\lambda)}} (1 - e^{Y-Y_{max}})$$

$$\mu_{max} = \mu_{opt} \left\{ \frac{(pH - pH_{min})(pH - pH_{max})}{(pH - pH_{min})(pH - pH_{max}) - (pH - pH_{opt})^2} \right\} \left\{ \frac{a_w - a_{wmin}}{1 - a_{wmin}} \right\}$$

and will be henceforth referred to as the Huang-Cardinal [pH] (Equation (1)) and Huang-Cardinal [pH, a_w] model (Equation (2)). Their ability to closely describe the experimental growth curves was compared. In Equations (1) and (2), Y_0 , Y_{max} and Y are the natural logarithms of bacterial counts at time 0, at maximum level and at the 'real time' t , respectively; μ_{max} is the specific growth rate (ln CFU/g h⁻¹); λ is the lag time (h) of a growth curve; α is the lag phase transition coefficient (dimensionless); and t is the time (h) under a constant temperature (7 °C ± 1.0). While λ was set to zero, α was given a value of 4.0, as recommended in Huang (2013). The terms pH_{min} and pH_{max} are the pH below or above which no growth occurs, while pH_{opt} is the pH at which the specific growth μ_{max} is optimal. Likewise, a_{wmin} is the water activity below which no growth occurs. The estimated parameters from Equations (1) and (2) were Y_0 , Y_{max} and μ_{opt} . The parameter μ_{opt} in Equation (1) can be interpreted as the optimum growth rate at 7°C of *L. monocytogenes* in Minas fresh cheese at the optimum pH (pH_{opt}), assuming that the variation in a_w during the short storage time of this cheese is negligible. The parameter μ_{opt} in Equation (2) is defined as the optimum growth rate at 7°C of *L. monocytogenes* in Minas fresh cheese at the optimum pH (pH_{opt}) and at the optimum a_w (a_{wopt} fixed at 1.0). Because the cardinal parameters of *L. monocytogenes* (pH_{min} , pH_{opt} , pH_{max} and a_{wmin}) are not estimable from the data – as the monitored pH (6.9–4.7) and a_w (0.999–0.993) of the Minas cheese correspond to narrow-ranged suboptimal values, they were set to the values estimated by Augustin et al. (2005) in liquid microbiological media: $pH_{min} = 4.71$ (condition where lactic acid is present); $pH_{opt} = 7.10$; $pH_{max} = 9.61$; $a_{wmin} = 0.913$.

2.4.2. Describing the growth of *L. monocytogenes* by dynamic competition models

The simultaneous growth of *L. monocytogenes* and autochthonous/selected LAB in cheese during refrigerated storage was described by three variants of the Jameson-effect model based on a logistic deceleration function. The logistic deceleration provides an empirical description of a self-limiting growth process, which represents the exhaustion of essential nutrients, the accumulation of waste products inhibiting growth and/or the lowering of pH due to acid production. In its simplest form, the Jameson-effect model assumes that LAB and *L. monocytogenes* inhibit each other to the same extent that they inhibit

their own growth, and that one microorganism stops growing when the other has reached its maximum density. Under these assumptions, the simple Jameson-effect model (Equation (3)) defined as,

$$\frac{1}{LM} \frac{dLM}{dt} = \mu_{LM} \left(1 - \frac{LM}{LM_{max}} \right) \left(1 - \frac{LAB}{LAB_{max}} \right)$$

$$\frac{1}{LAB} \frac{dLAB}{dt} = \mu_{LAB} \left(1 - \frac{LAB}{LAB_{max}} \right) \left(1 - \frac{LM}{LM_{max}} \right)$$

was fitted to each of the four sets of experimental growth curves. LM and LAB are the counts of *L. monocytogenes* and LAB bacteria in time while LM_{max} and LAB_{max} are their maximum population densities (ln CFU/g). The parameters μ_{LM} and μ_{LAB} are the maximum specific growth rates of *L. monocytogenes* and LAB (ln CFU/g h⁻¹), respectively.

A more flexible Jameson-effect model which includes an interaction parameter γ , allowing the inhibition effect of the natural microbiota on pathogens growth to differ among environmental conditions (Giménez and Dalgaard, 2004), was also fitted to the four sets of experimental curves.

$$\frac{1}{LM} \frac{dLM}{dt} = \mu_{LM} \left(1 - \frac{LM}{LM_{max}} \right) \left(1 - \frac{\gamma \times LAB}{LAB_{max}} \right)$$

$$\frac{1}{LAB} \frac{dLAB}{dt} = \mu_{LAB} \left(1 - \frac{LAB}{LAB_{max}} \right) \left(1 - \frac{LM}{LM_{max}} \right)$$

Equation (4) is referred to as the Jameson-effect model with γ , where the parameter γ allows the *L. monocytogenes* counts to increase after LAB has reached its maximum density ($\gamma < 1$), or to decrease after LAB has reached its maximum density ($\gamma > 1$).

The third Jameson-effect variant fitted to the simultaneous growth data was that of Cornu (2001) who proposed a modification of the logistic deceleration function by considering instead a total maximum cell density $N_{max\ tot}$.

$$\frac{1}{LM} \frac{dLM}{dt} = \mu_{LM} \left(1 - \frac{LM + LAB}{N_{max\ tot}} \right)$$

$$\frac{1}{LAB} \frac{dLAB}{dt} = \mu_{LAB} \left(1 - \frac{LAB + LM}{N_{max\ tot}} \right)$$

Equation (5) is referred to as the Jameson-effect model with $N_{max\ tot}$. Although the Jameson-effect models were initially proposed to be used in prediction or forward analysis (i.e., to simulate the kinetics of both populations in co-culture) on the basis of their growth parameters estimated in mono-culture; in this study, the models of Equations (3)–(5) were used in inverse analysis, i.e. to estimate the kinetic parameters of the two microbial populations from experimental growth curves obtained in co-culture.

The classical prey-predator Lotka-Volterra competition model, introduced by Vereecken et al. (2000), was also evaluated. This model assumes that the competition for a common substrate is described by two inhibition coefficients that must be estimated from the microbial growth curves in co-culture. The Lotka-Volterra primary model (Equation (6)) is the system with two inhibition functions,

$$\frac{1}{LM} \frac{dLM}{dt} = \mu_{LM} \left(1 - \frac{LM + \alpha_{LM-LAB} LAB}{LM_{max}} \right)$$

$$\frac{1}{LAB} \frac{dLAB}{dt} = \mu_{LAB} \left(1 - \frac{LAB + \alpha_{LAB-LM} LM}{LAB_{max}} \right)$$

where α_{LAB-LM} and α_{LM-LAB} are the inhibition or interaction coefficients measuring the effects of *L. monocytogenes* on LAB and of LAB on *L. monocytogenes*, respectively. If $\alpha_{LM-LAB} < 1$, the effect of LAB on *L. monocytogenes* is less than the effect of LAB on its own population. Conversely, if $\alpha_{LM-LAB} > 1$, the effect of LAB on *L. monocytogenes* is greater than the effect of LAB on its own population. For simplicity, it

was assumed that *L. monocytogenes* did not influence the growth of LAB apart from the carrying capacity of the medium (retarding the growth of both populations), and hence a value of one was set to $\alpha_{\text{LAB-LM}}$.

2.4.3. Estimation of parameters

Models 2 to 6 include ordinary differential equations (ODE) that do not have an analytical solution, but can be solved with numerical methods. Numerical optimization consists of searching for the most suitable parameters of the dynamic models such that the residual sum of squares (RSS) of the errors is minimized. The 4th order Runge-Kutta method was adopted to solve ODE (Huang, 2012) while the unknown kinetic parameters were estimated by least-square optimization, using the ‘deSolve’ and ‘FME’ libraries implemented in the R software. The kinetic parameters of Equation (1) were estimated using the ‘nlme’ library. The mean absolute error (MAE) and root mean square error (RMSE), defined as,

$$MAE = \frac{\sum |Y_{\text{obs}i} - Y_{\text{fit}i}|}{n}$$

$$RMSE = \sqrt{\frac{\sum (Y_{\text{obs}i} - Y_{\text{fit}i})^2}{df}}$$

were used to compare the dynamic models. The variance of the residuals was also calculated. $Y_{\text{fit}i}$ and $Y_{\text{obs}i}$ denote each of the i -th concentration of *L. monocytogenes* fitted by the dynamic/competition model and its corresponding observation, respectively. The degree of freedom (df) is calculated as ‘n-np’, where n is the number of observations of an experimental growth curve and np is the number of parameters of the fitted model. Notice that for the Jameson-effect models (where *L. monocytogenes* and LAB are modeled simultaneously), the residuals from the LAB counts were not taken into account for the calculation of these fitting measures in order to make them comparable with those of the Huang-Cardinal models, where the residuals are only based on *L. monocytogenes* counts.

3. Results

3.1. Describing the growth of *L. monocytogenes* by dynamic tertiary models

A comparison of ability of the dynamic tertiary models to describe the experimental growth curves is detailed in Table 1. Both the Huang-Cardinal [pH] and Huang-Cardinal [pH- a_w] models showed that the addition of selected LAB with anti-listerial activity to raw or pasteurized milk reduced the μ_{opt} (0.0256–0.0336 ln CFU/g h⁻¹) and Y_{max} (14.08–14.83 ln CFU/g) of *L. monocytogenes* in comparison to those cheeses without addition of selected LAB (μ_{opt} : 0.0368–0.0405 ln CFU/g h⁻¹ and Y_{max} : 17.76–14.91 ln CFU/g). These kinetic parameters were slightly lower in raw milk cheeses vs. in pasteurized milk. The cheese made of raw milk with selected LAB was the one among the four treatments that provided the most restrictive conditions for the growth of *L. monocytogenes*, since these media presented the lowest μ_{opt} (0.0256 and 0.0281 ln CFU/g h⁻¹) and Y_{max} (14.08 and 14.12 ln CFU/g), as modeled by both the Huang-Cardinal [pH] and Huang-Cardinal [pH- a_w] model, respectively (Table 1).

The treatment using pasteurized milk and no addition of LAB with anti-listerial activity favored the most the growth of *L. monocytogenes*, because, as shown in Fig. 1, the cheese pH remained high with little change throughout storage. A considerable pH drop in cheese without added LAB was obtained when milk was not pasteurized, because autochthonous LAB were able to acidify the cheese, resulting in a cheese pH of 5.75 after 360 h storage. A steeper pH drop was observed in cheeses formulated with selected LAB (as low as pH ~ 4.80 after 360 h). *L. monocytogenes* presented slower growth in Minas cheese made with raw milk than in cheese made with pasteurized milk (Fig. 1).

Estimates of Y_0 and Y_{max} by the Huang-Cardinal [pH] and the

Table 1

Kinetic parameters (initial and maximum microbial concentration, Y_0 , Y_{max} in ln CFU/g, and optimum growth rate, μ_{opt} in ln CFU/g h⁻¹) of *L. monocytogenes* in Minas soft cheese elaborated with raw or pasteurized milk and with addition or not of lactic acid bacteria with anti-listerial activity (LAB_{LM}), as estimated by dynamic tertiary modeling, along with goodness-of-fit measures (residuals, σ^2 , root mean square error, RMSE, and mean absolute error, MAE) for model comparison.

Treatment	Parameters	Huang-Cardinal [pH]		Huang-Cardinal [pH, a_w]	
		Mean (SE)	p-value	Mean (SE)	p-value
Pasteurized milk + No LAB _{LM}	Y_0	14.71 (0.319)	< .0001	14.71 (0.385)	< .0001
	μ_{opt}	0.0395 (0.0087)	0.001	0.0405 (0.0135)	0.0122
	Y_{max}	17.76 (0.213)	< .0001	17.76 (0.272)	< .0001
	Fit quality				
	σ^2	0.1673		0.1668	
	RMSE	0.3942		0.3955	
	MAE	0.3297		0.3228	
Pasteurized milk + LAB _{LM}	Y_0	13.16 (0.134)	< .0001	13.15 (0.142)	< .0001
	μ_{opt}	0.0289 (0.0054)	< .0001	0.0336 (0.0070)	0.0006
	Y_{max}	14.77 (0.106)	< .0001	14.83 (0.143)	< .0001
	Fit quality				
	σ^2	0.0268		0.0285	
	RMSE	0.1578		0.1627	
	MAE	0.1182		0.1202	
Raw milk + No LAB _{LM}	Y_0	14.58 (0.242)	< .0001	14.58 (0.241)	< .0001
	μ_{opt}	0.0368 (0.0066)	0.001	0.0389 (0.0068)	0.0010
	Y_{max}	17.91 (0.194)	< .0001	17.91 (0.193)	< .0001
	Fit quality				
	σ^2	0.0872		0.0862	
	RMSE	0.2800		0.2786	
	MAE	0.2401		0.2389	
Raw milk + LAB _{LM}	Y_0	13.11 (0.107)	< .0001	13.11 (0.115)	< .0001
	μ_{opt}	0.0256 (0.0050)	0.001	0.0281 (0.0067)	0.0029
	Y_{max}	14.08 (0.045)	< .0001	14.12 (0.072)	< .0001
	Fit quality				
	σ^2	0.0038		0.0039	
	RMSE	0.0589		0.0595	
	MAE	0.0529		0.0536	

Huang-Cardinal [pH- a_w] model were essentially equivalent, while μ_{opt} estimates by the Huang-Cardinal [pH- a_w] model were slightly higher than those of the Huang-Cardinal [pH] model. Optimum growth rates fitted by the Huang-Cardinal [pH- a_w] were higher because they were extrapolated to the scale of optimum water activity of 1.0, whereas the optimum growth rates fitted by Huang-Cardinal [pH] model were lower for being based on the suboptimal water activity range of the cheese during storage. Both dynamic models presented a good visual fit quality (fit only shown for the Huang-Cardinal [pH] model in Fig. 2), however treatments with addition of anti-listerial LAB had the lowest goodness-of-fit values; suggesting that the measurements taken from cheeses with autochthonous LAB were associated to greater variability (Table 1). Within each cheese type, values of residuals (σ^2), root mean square

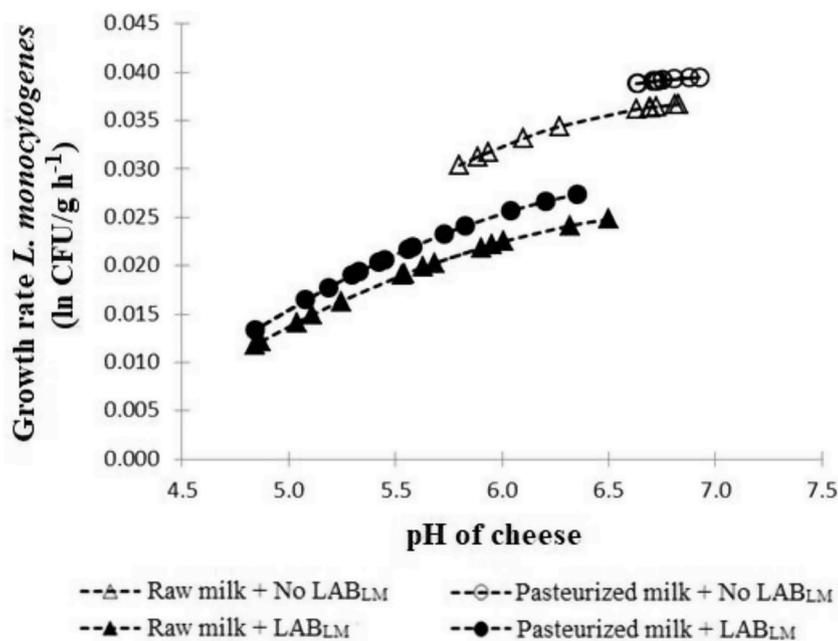


Fig. 1. Effect of pH on the specific growth rate of *L. monocytogenes* in Minas soft cheese elaborated with raw or pasteurized milk and with addition or not of lactic acid bacteria with anti-listerial activity (LAB_{LM}), as depicted by the cardinal parameter model [pH]. Markers indicate the time points at which cheese pH were measured from time 0 (rightmost marker) until 360 h (leftmost marker) of refrigerated storage.

error (RMSE) and mean absolute error (MAE) were comparable between the dynamic models. This arises because the incorporation of a_w evolution in the tertiary dynamic model is not very useful in a fresh cheese whose a_w spans only from 0.999 to 0.993, since the change in growth rate of *L. monocytogenes* is primarily driven by the drop in pH.

Maximum population density of *L. monocytogenes* in raw milk Minas cheese with selected LAB was estimated to be 14.12 ln CFU/g, with an optimum growth rate of 0.0281 ln CFU/h considering an optimum pH of 7.1 and a_w of 1.0 when a more comprehensive model (Huang-Cardinal [pH- a_w]) was used.

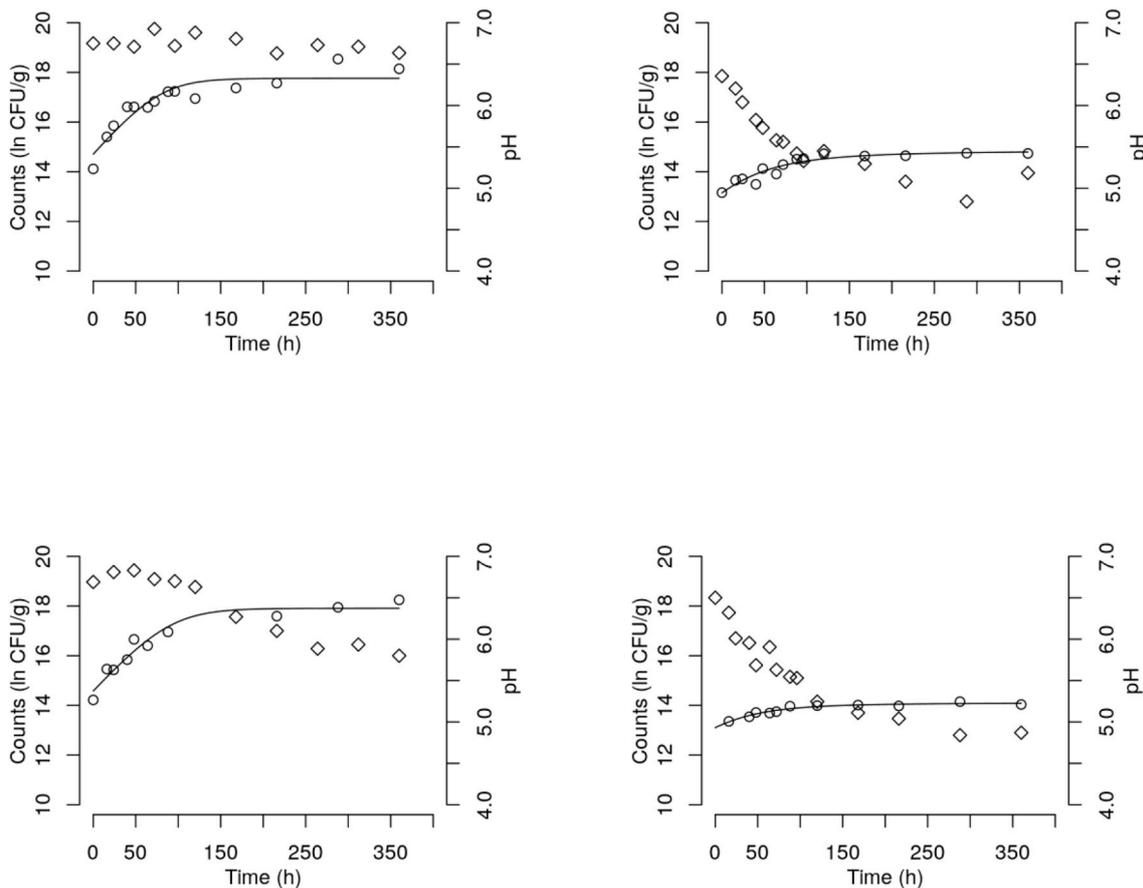


Fig. 2. Evolution of pH (◊) and growth of *L. monocytogenes* (○) in Minas soft cheese elaborated from pasteurized milk without addition of LAB (top left), pasteurized milk with addition of LAB (top right), raw milk without addition of LAB (bottom left) and raw milk with addition of LAB (bottom right), as depicted by the Huang-Cardinal [pH] model.

Table 2

Kinetic parameters (initial and maximum microbial concentrations, LAB_0 , LM_0 , LAB_{max} and LM_{max} [ln CFU/g], and optimum growth rates, μ_{LAB} and μ_{LM} [ln CFU/g h^{-1}]) of lactic acid bacteria (LAB) and *L. monocytogenes* in Minas soft cheese elaborated with raw or pasteurized milk and with addition or not of LAB with anti-listerial activity (LAB_{LM}), as estimated by the simple Jameson-effect and the Jameson-effect with γ (interaction parameter), along with goodness-of-fit measures (residuals, σ^2 , root mean square error, RMSE, and mean absolute error, MAE) computed for *L. monocytogenes* counts only to allow comparison.

Treatment	Parameters	Simple Jameson-effect		Jameson-effect with γ	
		Mean (SE)	p-value	Mean (SE)	p-value
Pasteurized milk + No LAB_{LM}	LAB_0	6.193 (0.373)	< .0001	6.403 (0.396)	< .0001
	LM_0	14.34 (0.254)	< .0001	14.48 (0.381)	< .0001
	LAB_{max}	17.56 (0.262)	< .0001	17.11 (0.240)	< .0001
	LM_{max}	17.52 (0.140)	< .0001	17.98 (0.957)	< .0001
	μ_{LAB}	0.2252 (0.0189)	< .0001	0.2031 (0.0258)	< .0001
	μ_{LM}	0.0633 (0.0050)	< .0001	0.0489 (0.0171)	0.0010
	γ	–	–	0.9863 (0.0105)	< .0001
	Fit quality				
	σ^2	0.1867		0.0975	
	RMSE	0.4187		0.3070	
MAE	0.3064		0.2556		
Pasteurized milk + LAB_{LM}	LAB_0	17.57 (0.362)	< .0001	17.56 (0.583)	< .0001
	LM_0	13.09 (0.291)	< .0001	12.98 (0.174)	< .0001
	LAB_{max}	25.60 (6.296)	< .0001	25.06 (0.626)	< .0001
	LM_{max}	15.09 (0.286)	< .0001	15.34 (0.678)	< .0001
	μ_{LAB}	0.0540 (0.0103)	< .0001	0.0517 (0.0206)	0.0201
	μ_{LM}	0.0151 (0.0034)	0.0002	0.0183 (0.0017)	< .0001
	γ	–	–	1.0388 (0.0096)	< .0001
	Fit quality				
	σ^2	0.0784		0.1340	
	RMSE	0.3250		0.4645	
MAE	0.2788		0.3347		
Raw milk + No LAB_{LM}	LAB_0	10.58 (0.302)	< .0001	10.73 (0.274)	< .0001
	LM_0	14.92 (0.246)	< .0001	14.61 (0.266)	< .0001
	LAB_{max}	18.51 (0.454)	< .0001	18.47 (0.612)	< .0001
	LM_{max}	17.84 (0.229)	< .0001	17.93 (0.249)	< .0001
	μ_{LAB}	0.0957 (0.0109)	< .0001	0.0888 (0.0085)	< .0001
	μ_{LM}	0.0354 (0.0043)	< .0001	0.0367 (0.0071)	0.0001
	γ	–	–	0.9865 (0.0531)	< .0001
	Fit quality				
	σ^2	0.1358		0.0809	
	RMSE	0.3531		0.2739	
MAE	0.2935		0.2214		
Raw milk + LAB_{LM}	LAB_0	19.82 (0.335)	< .0001	19.59 (0.525)	< .0001
	LM_0	13.15 (0.238)	< .0001	13.28 (0.515)	< .0001
	LAB_{max}	22.89 (0.679)	< .0001	22.89 (1.014)	< .0001
	LM_{max}	14.19 (0.197)	< .0001	14.14 (0.280)	< .0001
	μ_{LAB}	0.0523 (0.0186)	0.0117	0.0540 (0.0207)	0.0200
	μ_{LM}	0.0184 (0.0068)	0.0151	0.0176 (0.0046)	0.0018
	γ	–	–	1.0143 (0.0620)	< .0001
	Fit quality				
	σ^2	0.0045		0.0058	
	RMSE	0.0792		0.0796	
MAE	0.0563		0.0594		

3.2. Describing the growth of *L. monocytogenes* by dynamic competition models

The Jameson-effect model parameters for simultaneous growth of *L. monocytogenes* and LAB in soft cheese during refrigerated shelf-life are shown in Table 2 (simple Jameson-effect and Jameson-effect with interaction γ) and Table 3 (Jameson with $N_{max\ tot}$). The three variants appeared statistically adequate to depict the simultaneous microbial growth of *L. monocytogenes* and LAB in each of the cheese treatments, since all the parameters were significant. The maximum concentrations of LAB and *L. monocytogenes* estimated from the simple and the γ -Jameson effect were very close. The three Jameson-effect models all estimated that treatments without addition of anti-listerial LAB presented higher values of growth rates for LAB and *L. monocytogenes*, resulting in

a shorter time to reach the maximum concentrations of these microorganisms, which can also be observed in Figs. 2–5. The fact that, in cheeses with added LAB, the initial concentration of LAB is closer to the maximum carrying capacity slowed down their growth, regardless of the type of milk used. Moreover, the *L. monocytogenes* growth rate estimates from cheeses with added anti-listerial LAB should be carefully interpreted, in particular for the experiment in raw milk; since they were associated with greater coefficients of variation (CV) (i.e., CV of μ_{LM} for raw milk + No LAB_{LM} : $0.0043/0.0354 = 0.121$ while for raw milk + LAB_{LM} : $0.0068/0.0184 = 0.369$). The fact of having only marginal increases in *L. monocytogenes* counts when anti-listerial LAB was added to raw or pasteurized milk affected the optimization of the kinetic parameters in both cases. In addition to this, the correlations among the competition model's parameters made the optimization

Table 3

Kinetic parameters (initial and maximum microbial concentrations, LAB_0 , LM_0 , LAB_{max} and LM_{max} [ln CFU/g], and optimum growth rates, μ_{LAB} and μ_{LM} [ln CFU/g h^{-1}]) of lactic acid bacteria (LAB) and *L. monocytogenes* in Minas soft cheese elaborated with raw or pasteurized milk and with addition or not of LAB with anti-listerial activity (LAB_{LM}), as estimated by the Jameson-effect with $N_{tot\ max}$ and the simplified Lotka-Volterra, along with goodness-of-fit measures (residuals, σ^2 , root mean square error, RMSE, and mean absolute error, MAE) computed for *L. monocytogenes* counts only to allow comparison.

Treatment	Parameters	Jameson-effect with $N_{tot\ max}$		Simplified Lotka-Volterra		
		Mean (SE)	p-value	Mean (SE)	p-value	
Pasteurized milk + No LAB_{LM}	LAB_0	6.519 (0.442)	< .0001	6.378 (0.462)	< .0001	
	LM_0	14.67 (0.366)	< .0001	14.39 (0.436)	< .0001	
	LAB_{max}	–	–	17.97 (0.135)	< .0001	
	LM_{max}	–	–	17.83 (0.451)	< .0001	
	μ_{LAB}	0.2009 (0.0192)	< .0001	0.2061 (0.0210)	< .0001	
	μ_{LM}	0.0524 (0.0079)	< .0001	0.0625 (0.0186)	0.0029	
	$N_{tot\ max}$	18.00 (0.133)	< .0001	–	–	
	$\ln(\alpha_{LM-LAB})$	–	–	–0.3627 (1.1446)	0.7544	
	Fit quality					
	σ^2	0.2408		0.2531		
	RMSE	0.4729		0.4863		
MAE	0.3428		0.3634			
Pasteurized milk + LAB_{LM}	LAB_0	18.02 (0.302)	< .0001	18.31 (0.957)	< .0001	
	LM_0	13.54 (0.271)	< .0001	13.35 (0.647)	< .0001	
	LAB_{max}	–	–	24.95 (1.859)	< .0001	
	LM_{max}	–	–	18.45 (2.466)	< .0001	
	μ_{LAB}	0.0339 (0.0042)	< .0001	0.0211 (0.004)	< .0001	
	μ_{LM}	0.0078 (0.0026)	0.0066	0.0102 (0.008)	0.2615	
	$N_{tot\ max}$	24.66 (0.416)	< .0001	–	–	
	$\ln(\alpha_{LM-LAB})$	–	–	–5.515 (2.553)	0.0425	
	Fit quality					
	σ^2	0.0689		0.1395		
	RMSE	0.2831		0.3740		
MAE	0.2216		0.2806			
Raw milk + No LAB_{LM}	LAB_0	10.85 (0.267)	< .0001	10.83 (0.292)	< .0001	
	LM_0	14.58 (0.237)	< .0001	14.60 (0.944)	< .0001	
	LAB_{max}	–	–	18.77 (0.178)	< .0001	
	LM_{max}	–	–	18.45 (2.183)	0.0150	
	μ_{LAB}	0.0788 (0.0066)	< .0001	0.0792 (0.0073)	< .0001	
	μ_{LM}	0.0350 (0.0042)	< .0001	0.0346 (0.0593)	0.5700	
	$N_{tot\ max}$	18.77 (0.160)	< .0001	–	–	
	$\ln(\alpha_{LM-LAB})$	–	–	–0.6667 (27.63)	0.9810	
	Fit quality					
	σ^2	0.1022		0.0901		
	RMSE	0.3157		0.2848		
MAE	0.2628		0.2448			
Raw milk + LAB_{LM}	LAB_0	19.87 (0.366)	< .0001	19.74 (0.459)	< .0001	
	LM_0	13.29 (0.254)	< .0001	13.23 (0.307)	< .0001	
	LAB_{max}	–	–	22.40 (0.200)	< .0001	
	LM_{max}	–	–	17.05 (2.403)	< .0001	
	μ_{LAB}	0.0265 (0.0079)	0.0039	0.0310 (0.0112)	0.0145	
	μ_{LM}	0.0079 (0.0041)	0.0680	0.0103 (0.0058)	0.0987	
	$N_{tot\ max}$	22.50 (0.186)	< .0001	–	–	
	$\ln(\alpha_{LM-LAB})$	–	–	–5.359 (2.529)	0.0512	
	Fit quality					
	σ^2	0.0081		0.0045		
	RMSE	0.1601		0.0643		
MAE	0.1400		0.0055			

process slower and prone to instabilities, in particular for the γ -Jameson effect and the Lotka-Volterra competition models. Further research should look into the utilization of both lower concentrations of anti-listerial LAB and optimized sampling as a way to decrease the uncertainty about the μ_{LM} estimates.

The γ values for the treatments with anti-listerial LAB addition were slightly higher than 1, indicating that *L. monocytogenes* population decreases slightly after LAB reached its maximum density, and that LAB present an inhibitory effect on *L. monocytogenes* growth. In cheeses where there was no addition of selected LAB, γ values were less than 1,

suggesting that *L. monocytogenes* were not inhibited by LAB after reaching their maximum carrying capacity. Notice in Fig. 3 (top right) that the γ -Jameson-effect equation effectively modeled the slight increase of *L. monocytogenes* counts after LAB had reached the stationary phase. In those treatments where $\gamma < 1$ (i.e., no addition of selected LAB), the γ -Jameson-effect model yielded a better fit (i.e., lower goodness-of-fit measures) of the *L. monocytogenes* growth than the simple Jameson-effect, the Jameson-effect with $N_{max\ tot}$ or the tertiary dynamic models.

The $N_{max\ tot}$ -Jameson-effect model was the simplest Jameson-effect

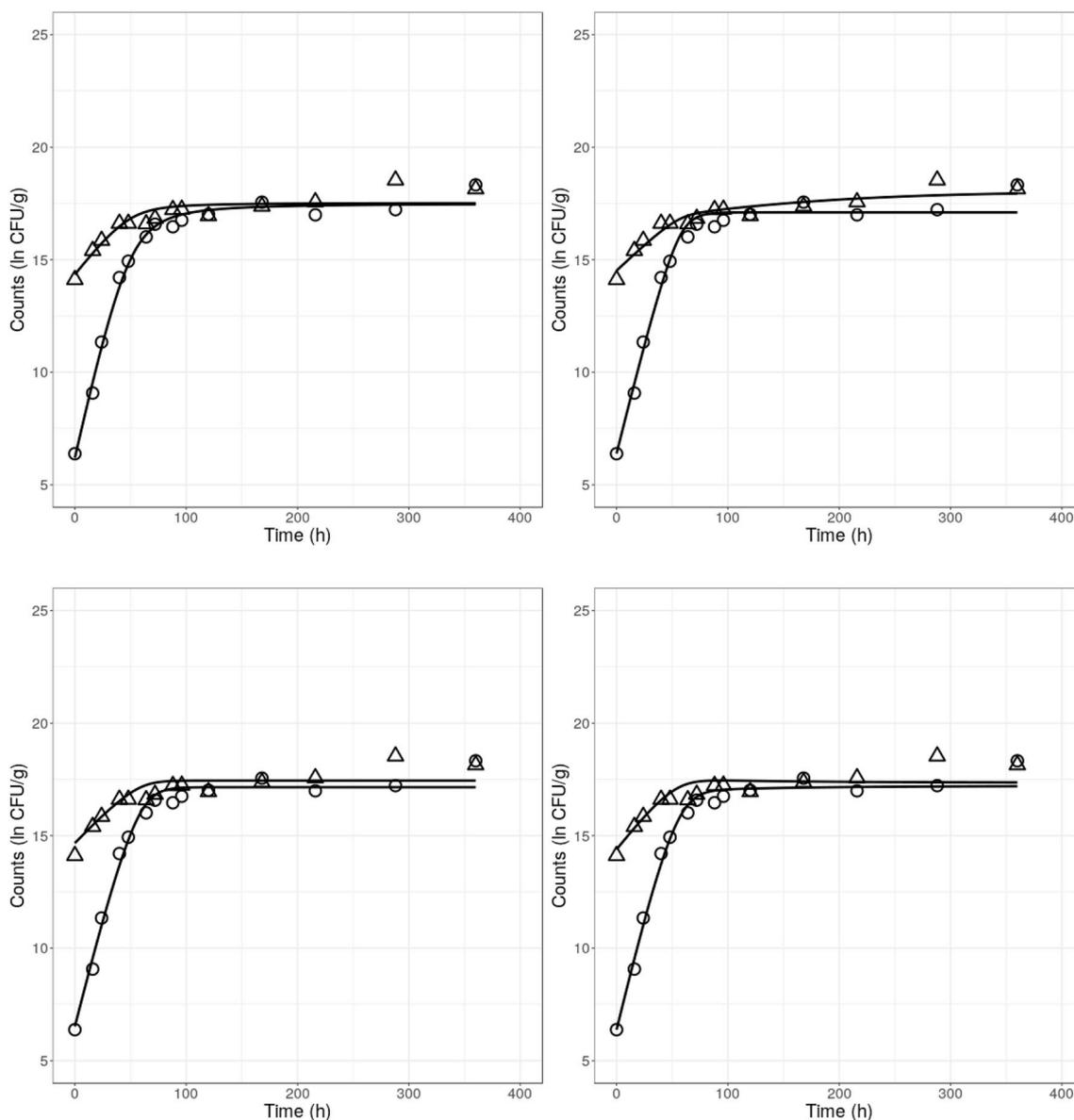


Fig. 3. Simultaneous growth of lactic acid bacteria (LAB, -O-) and *L. monocytogenes* (-Δ-) in Minas soft cheese elaborated from pasteurized milk without addition of LAB with anti-listerial activity, as depicted by the simple Jameson-effect (top left), Jameson-effect with γ (top right), Jameson effect with $N_{\max \text{ tot}}$ (bottom left) and Lotka-Volterra (bottom right) competition models.

model to fit with the fewest parameters to optimize (i.e., five as opposed to the six parameters of the simple Jameson-effect or the seven parameters of the γ -Jameson effect model). Although the goodness-of-fit parameters of the *L. monocytogenes* counts were sometimes higher compared to the other models, the $N_{\max \text{ tot}}$ -Jameson-effect model provided a good representation of the simultaneous growth of *L. monocytogenes* and LAB (Figs. 2–5). The $N_{\max \text{ tot}}$ -Jameson-effect model provided the lowest estimates of growth rates for *L. monocytogenes* (0.0079 or 0.0078 $\ln \text{ CFU h}^{-1}$, respectively) and LAB (0.0265 or 0.0339 $\ln \text{ CFU h}^{-1}$, respectively), in the cheeses formulated with anti-listerial LAB, regardless of using raw or pasteurized milk, which were about half the estimates of μ_{LM} (0.0176–0.0184 or 0.0151–0.0183 $\ln \text{ CFU h}^{-1}$, respectively) and μ_{LAB} (0.0523–0.0540 or 0.0517–0.0540 $\ln \text{ CFU h}^{-1}$, respectively) obtained by the other two Jameson-effect variants (Tables 2 and 3).

The Lotka-Volterra model depicted the *L. monocytogenes* growth better in the treatments using raw milk cheese, as per analysis of the goodness-of-fit measures (Table 3). The Lotka-Volterra model also generally presented the poorest fit among the four competition models

in pasteurized cheeses. We suggest interesting findings from the Lotka-Volterra model fits. Firstly, in cheeses where no anti-listerial LAB were added, the inhibition coefficients $\ln(\alpha_{LM-LAB})$ (viz. measuring effect of LAB on *L. monocytogenes*) were not significantly different from zero. Hence, if α_{LM-LAB} is equal to one, we deduce that there is no measurable inhibitory effect of autochthonous LAB on *L. monocytogenes*. If α_{LM-LAB} and α_{LAB-LM} in the Lotka-Volterra model (Equation (6)) are equal to one, the model becomes useless, and hence other competition models are more suitable for the Minas cheeses with indigenous LAB. The unsuitability of the Lotka-Volterra model can be also visually inferred from the fact that both LAB and *L. monocytogenes* population reach their maximum concentrations nearly at the same time in the raw milk cheese (Fig. 3, bottom left) and in the pasteurized milk cheese (Fig. 5, bottom left). Secondly, the parameter $\ln(\alpha_{LM-LAB})$ was significant in raw milk cheese (-5.359 ; $p = 0.05$) and in pasteurized milk cheese (-5.515 ; $p = 0.04$) for treatments with anti-listerial LAB, resulting in α_{LM-LAB} values of 0.005 and 0.004, respectively. Since these are values lower than one, the effect of LAB on *L. monocytogenes* is less than the effect of LAB on itself, although it is sufficient to produce a

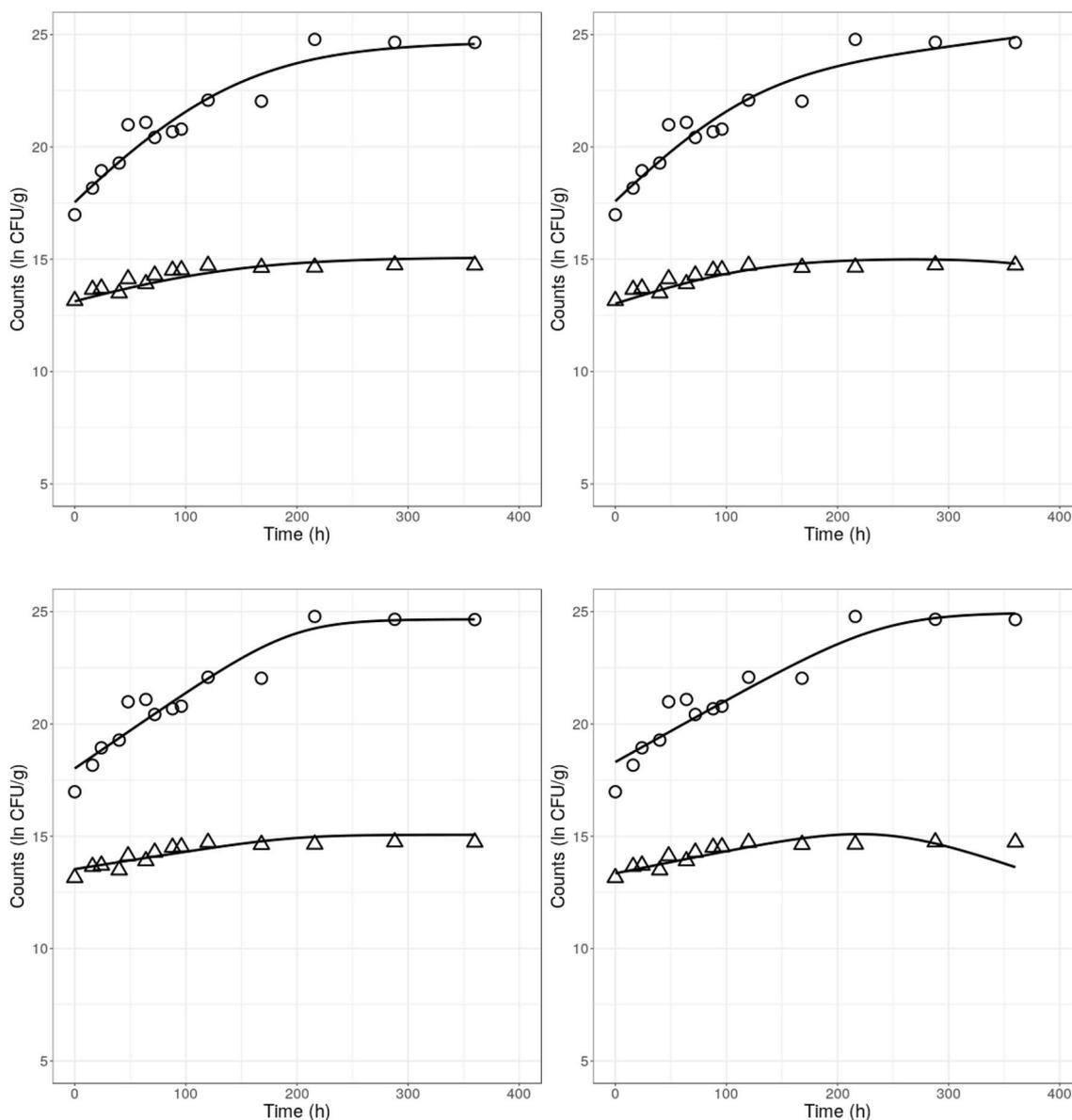


Fig. 4. Simultaneous growth of lactic acid bacteria (LAB, \circ -) and *L. monocytogenes* (Δ -) in Minas soft cheese elaborated from pasteurized milk with addition of LAB with anti-listerial activity, as depicted by the simple Jameson-effect (top left), Jameson-effect with γ (top right), Jameson effect with $N_{\max \text{ tot}}$ (bottom left) and Lotka-Volterra (bottom right) competition models.

bacteriostatic effect on *L. monocytogenes* (Figs. 4 and 6).

3.3. Comparison between dynamic tertiary and competition modeling

Based on (i) the goodness-of-fit quality measures (i.e., residuals, MAE and RMSE compiled in all Tables), and (ii) ease of parameter optimization; modeling the kinetics of *L. monocytogenes* as a function of the evolving pH (or as a function of the evolving pH and water activity) was more accurate than as a function of the inhibitory effect of LAB; in particular, the tertiary Huang-Cardinal [pH] model outperformed the simple Jameson-effect, the Jameson effect with $N_{\max \text{ tot}}$ and the Lotka-Volterra models. The γ -Jameson-effect model produced a slightly better representation of the experimental growth curves than the tertiary Huang-Cardinal [pH] model only in the treatments without added anti-listerial LAB. However, the decrease in the goodness-of-fit measures attained by the γ -Jameson-effect model was so marginal that it may not justify opting for such a more complex model when it comes to describing the dynamic growth of a pathogen in a *fermenting food* (i.e., with changes in pH). Contrasting the fit of the Huang-Cardinal model

(Fig. 2) to that of the competition models (Figs. 3–5), it became evident that the former depicted more accurately the transition from the exponential to the stationary phase, due to the transition function accommodated in the Huang primary growth model. In most cases, the maximum population density values fitted by the Huang-Cardinal model were slightly higher than those fitted by the competition models, and overall described better the stationary phase data.

It is equally important to note that the growth rates extracted from the tertiary and the competition models have different meanings. The pathogen's growth rate is an optimum rate in the tertiary models as it is based upon an optimum pH and water activity, while the pathogen's growth rate estimated from a microbial competition model is a rate constant, and, at least in this study, not linked to environmental factors. Nonetheless, a microbial competition model, which is in essence a primary model, could still be coupled to secondary models depicting the influence of an environmental factor; for instance, to assess the effect of storage temperature on LAB and *L. monocytogenes* growth rates. To this respect, it is worth mentioning that, in this study, all the parameters extracted from both the dynamic tertiary and the

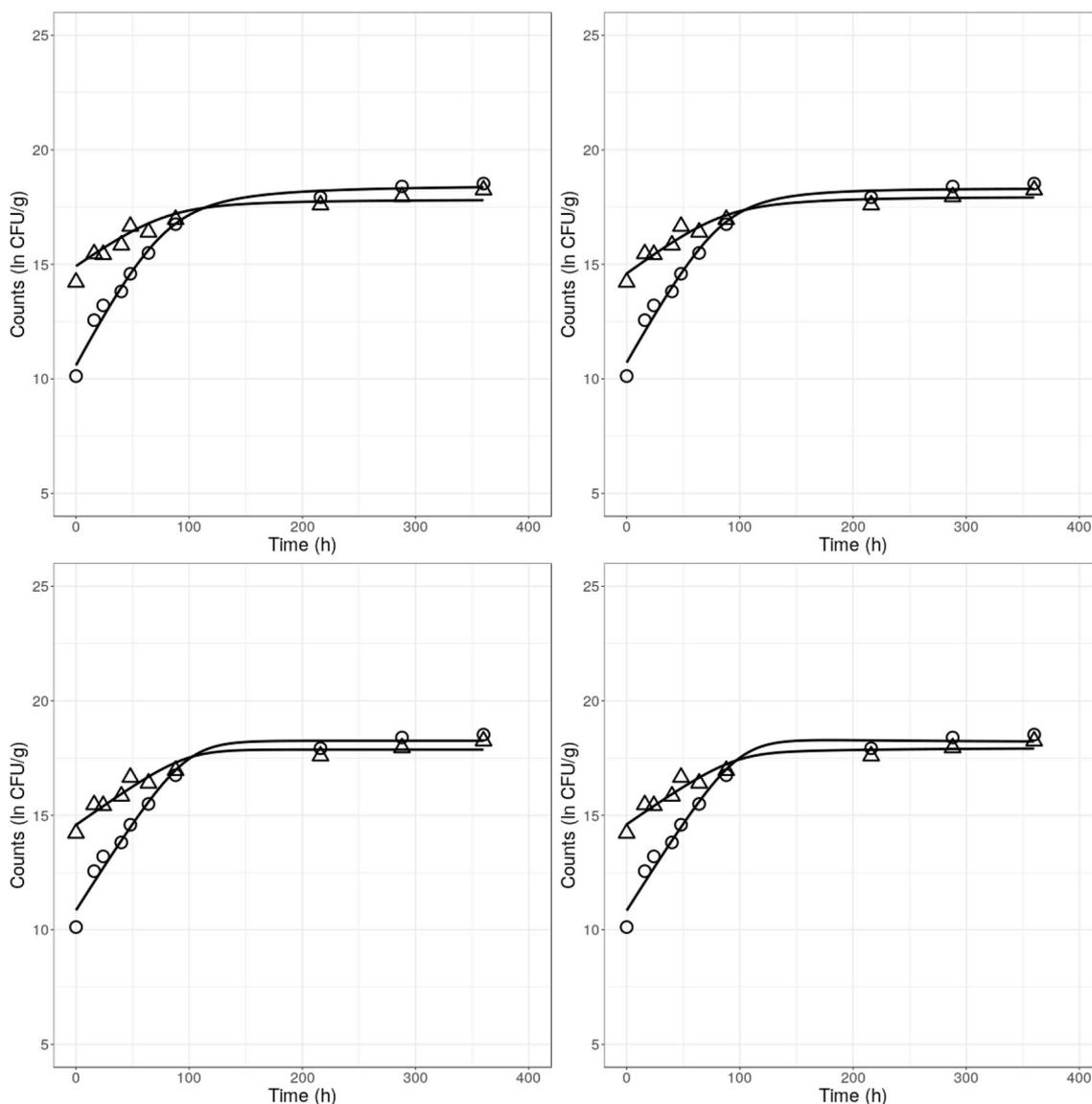


Fig. 5. Simultaneous growth of lactic acid bacteria (LAB, \circ) and *L. monocytogenes* (Δ) in Minas soft cheese elaborated from raw milk without addition of LAB with anti-listerial activity, as depicted by the simple Jameson-effect (top left), Jameson-effect with γ (top right), Jameson effect with $N_{\max \text{ tot}}$ (bottom left) and Lotka-Volterra (bottom right) competition models.

competition models characterize growth kinetics of *L. monocytogenes* and LAB at a constant storage temperature of 7 °C. Since the growth models, as defined in Equations (1)–(6), do not cope with temperature effect, they cannot be used to estimate kinetic parameters at other temperatures. Competition models can be still appropriate when the aim is to characterize the kinetic parameters of LAB in co-culture experiments.

4. Discussion

The primary model developed by Huang (2008), a growth model based on the classical bacterial growth process including lag, exponential and stationary phases, was associated to the cardinal parameter model in order to describe the *L. monocytogenes* growth based on the variation of environmental parameters such as pH and a_w . Since Minas soft cheese is a high moisture product and variation in a_w during refrigerated shelf-life is quite low, there was little or almost no difference between the models evaluated, the Huang-Cardinal [pH] and the Huang-Cardinal [pH- a_w], showing that this intrinsic parameter is not necessary for dynamic modeling of this fresh cheese. The Huang (2008)

model was originally developed from broth and beef frankfurters experiments, but by coupling a cardinal parameter model, we showed that it accurately describes the growth of *L. monocytogenes* in fresh cheese (Table 1). Models predicting the development over time of the bacterial population in cheese must be able to deal with the dynamic environment of this food matrix in order to obtain accurate estimates, particularly the dynamics of the most important driving factors such as the variation of pH over time according to Rosshaug et al. (2012). Rosso et al. (1995) have also emphasized that models should have biological meaning, a minimum number of parameters, easy applicability and good quality of fit. Based on these premises and on the fact that the addition of a_w parameter to the Huang-Cardinal model did not improve significantly the fit quality, the Huang-Cardinal [pH] model is good enough, and the best choice to describe *L. monocytogenes* growth in Minas fresh cheese.

The increased pH drop seen from the addition of LAB with anti-listerial activity is not unexpected since these bacteria were previously selected by Campagnollo et al. (2018) based on their acidifying and proteolytic capacity. The pH drop caused the reduction of specific growth rate of *L. monocytogenes* for all cheese treatments (Fig. 1), which

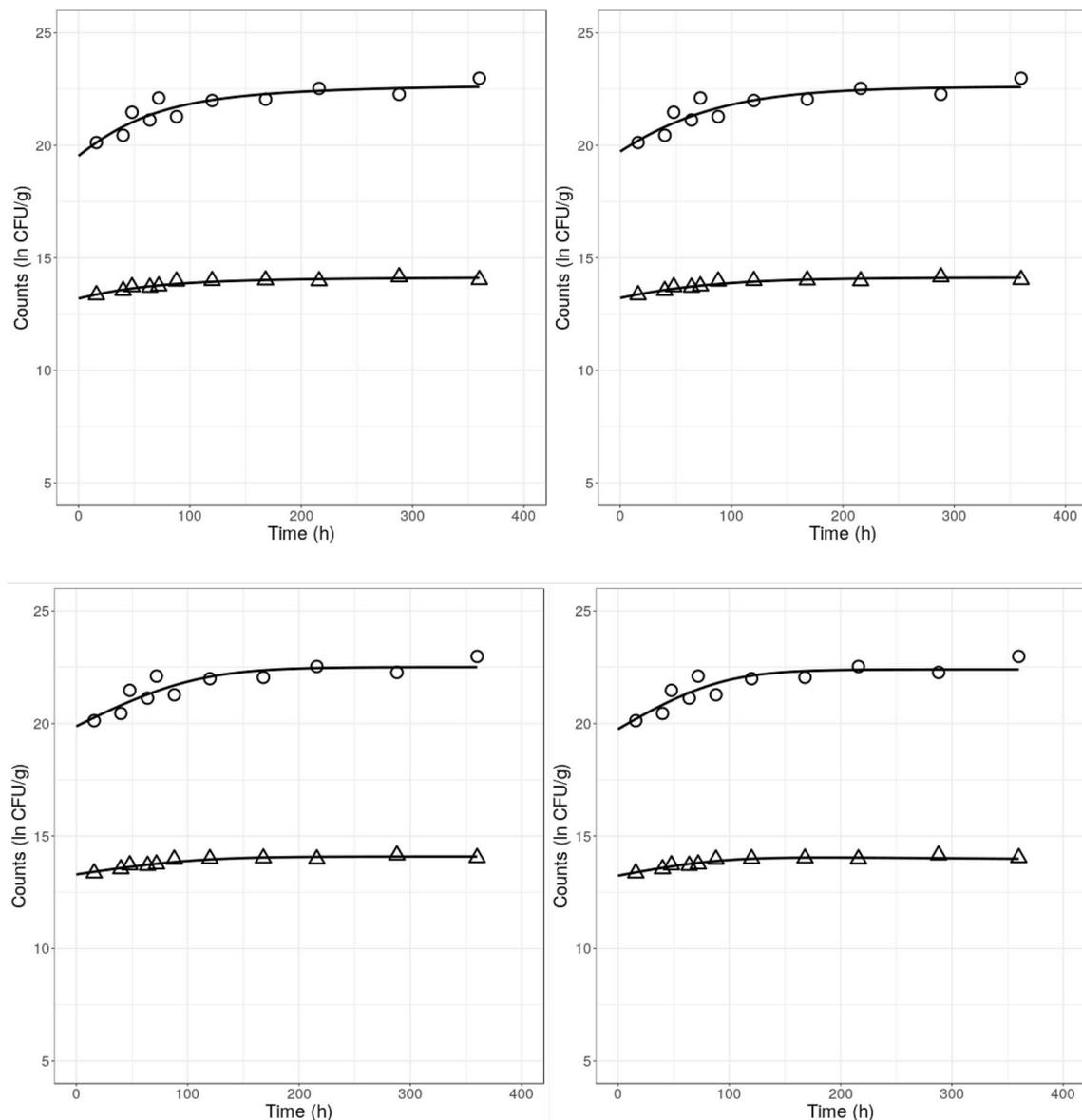


Fig. 6. Simultaneous growth of lactic acid bacteria (LAB, -O-) and *L. monocytogenes* (-Δ-) in Minas soft cheese elaborated from raw milk with addition of LAB with anti-listerial activity, as depicted by the simple Jameson-effect (top left), Jameson-effect with γ (top right), Jameson effect with $N_{\max \text{ tot}}$ (bottom left) and Lotka-Volterra (bottom right) competition models.

is consistent with the observations of [Wemmenhove et al. \(2018\)](#), who noted that lower cheese pH values result in increased concentrations of undissociated acids which have *L. monocytogenes* growth-inhibiting effects. [Ribeiro et al. \(2006\)](#) investigated the growth responses of persistent strains of *L. monocytogenes* isolated from cheeses to NaCl (5–10%) and pH (4.0–8.5) and also observed that higher specific growth rates were predicted for more alkaline pH values at relatively low salt concentrations. The ability of *L. monocytogenes* to overcome stresses from pH and a_w is a critical characteristic enabling its proliferation in cheese ([Melo et al., 2015](#)).

Our dynamic tertiary model to estimate the growth of *L. monocytogenes* considering environmental parameters in this Brazilian traditional soft cheese represents an important advance. Prior studies have evaluated only the presence or absence of the pathogen in Minas frescal cheese ([Brito et al., 2008](#); [Pinto et al., 2011](#); [Silva et al., 1998](#)) or the capacity of a compound or microorganism to inhibit its growth during processing or storage ([Malheiros et al., 2012](#); [Naldini et al., 2009](#); [Nascimento et al., 2008](#); [Silva et al., 2014](#)). [Campagnollo et al. \(2018\)](#) have modeled the fate of *L. monocytogenes* and LAB with anti-listerial

activity in Minas soft cheese using only primary models ([Baranyi and Roberts, 1994](#)). Few tertiary models have been used to predict *L. monocytogenes* growth in other cheeses. [Rosshaug et al. \(2012\)](#) developed a predictive dynamic tertiary model of *L. monocytogenes* growth in a soft blue-white cheese as a function of temperature, pH, NaCl and lactic acid, while [Schvartzman et al. \(2011\)](#) used a logistic primary model coupled with a secondary cardinal model taking into account environmental parameters such as temperature, pH, a_w and lactate content to analyze the *L. monocytogenes* behavior in smeared cheese made with raw or pasteurized milk. Both groups concluded that such models had important limitations as inactivation could not be predicted, and that the quality of the predictions depended on the period of the ripening for which predictions were done.

Studies considering the growth or inactivation of *L. monocytogenes* in cheese are quite common, but few have analyzed the influence of microbial interactions. [Guillier et al. \(2008\)](#) and [Augustin et al. \(2005\)](#) both noted that *L. monocytogenes* growth was sometimes incorrectly predicted in cheese as microbial interactions were not taken into account by the growth boundary models. Inhibition of *L. monocytogenes*

by LAB may occur by substrate competition or product inhibition including bacteriocins, peptides, organic acids, fatty acids, volatile compounds, H₂O₂ and interaction between these factors (Østergaard et al., 2014). Irlinger and Mounier (2009) stated that prediction of *L. monocytogenes* growth in cheese and other fermented dairy products can be a challenging task because it demands an understanding of the cheese microbial ecology to produce a product with a constant quality and safety. Guillier et al. (2008) modeled the competitive growth between *L. monocytogenes* and biofilm microflora of a soft and smear cheese wooden shelves using the Jameson-effect model and concluded that the inhibition of *L. monocytogenes* by the natural biofilm microorganisms is a result of a non-specific competition for nutrients. Østergaard et al. (2014) developed four mathematical models for evaluation of simultaneous growth of mesophilic LAB cultures and *L. monocytogenes* during chilled storage of cottage cheese, and the Jameson approach was also used. The simple Jameson-effect model or its variants have been used in the characterization of the simultaneous growth of LAB and *L. monocytogenes* or other microorganisms in distinct foods (Giménez and Dalgaard, 2004; Le Marc et al., 2009; Mejilholm and Dalgaard, 2007, 2015; Møller et al., 2013).

Although the Lotka-Volterra model has been commonly used to quantify the competitive growth between microorganisms in different foods, there is no previous work considering this model for measuring the *L. monocytogenes* and LAB interactions in cheese. Giuffrida et al. (2009) used a modified version of the Lotka-Volterra model to stochastically simulate the behavior of *L. monocytogenes* and LAB during the fermentation period of a typical Sicilian salami and concluded that the choice of a suitable parameter accounting for the interaction of LAB on *L. monocytogenes* as well as the introduction of appropriate noise levels allows good fits for both for the mean growth curves and for probability distributions of *L. monocytogenes* concentrations. Ye et al. (2014) studied the competitive growth of *L. monocytogenes* and *Lactobacillus* on vacuum-packaged chilled pork using a modified version of Lotka-Volterra model and observed that the influence of *Lactobacillus* on *L. monocytogenes* was much higher than that of *L. monocytogenes* on *Lactobacillus* through the interaction coefficients. Mounier et al. (2008) used the Lotka-Volterra model as a preliminary approach to represent inter- and intraspecies interactions between LAB and yeast in cheese. This model succeeded in representing the growth of the different microbial populations, resulting in negative yeast-yeast interactions and positive yeast-bacterium interactions.

5. Conclusion

Both the dynamic tertiary models based on the Huang-Cardinal equation and the microbial competition models – including Jameson-effect variants and Lotka-Volterra – were capable of closely describing the growth of *L. monocytogenes* in Minas fresh cheese during refrigerated storage. Incorporating a_w evolution in the tertiary Huang-Cardinal [pH] model was not necessary since the a_w of the Minas fresh cheese varied only slightly from 0.999 to 0.993 in 360 h; and any the change in growth rate of *L. monocytogenes* was primarily driven by the drop in pH. The tertiary Huang-Cardinal [pH] model outperformed the simple Jameson-effect, the Jameson effect with $N_{\max \text{ tot}}$ and the Lotka-Volterra models. The γ -Jameson-effect model produced a slightly better representation of the experimental growth curves than the tertiary Huang-Cardinal [pH] model. Only in treatments without added anti-listerial LAB; although the improvement in fit was only marginal considering the additional parameters to optimize. Modeling the kinetics of *L. monocytogenes* as a function of the evolving pH was more accurate than considering the inhibitory effect of LAB. Competition models could still be used if the aim was to simultaneously characterize the kinetic parameters of LAB from a co-culture experiment.

The γ -Jameson-effect model described *L. monocytogenes* growth in cheeses without anti-listerial LAB; while the Lotka-Volterra model estimated an interaction parameter $\alpha_{\text{LM-LAB}}$ that was significant only in

cheeses containing added anti-listerial LAB. The low values of such parameters ($\ln(\alpha_{\text{LM-LAB}}) = -5.359$ to -5.515) imply that the effect of LAB on *L. monocytogenes* was less than the effect of LAB on its own population. Likewise, the γ -Jameson-effect model, when fitted to the treatments with anti-listerial LAB, produced γ values slightly higher than one, suggesting that *L. monocytogenes* population could decrease after LAB had reached their maximum density. Both the Lotka-Volterra and γ -Jameson-effect competition models showed the bacteriostatic/inhibitory effect on the selected LAB on *L. monocytogenes* growth, and both models offered the possibility to quantitatively characterize the bacteriostatic/inhibitory effect of the selected LAB with anti-listerial activity in Minas fresh cheese. While the Jameson-effect with $N_{\max \text{ tot}}$ model was the simplest to fit (i.e. five parameters) among the microbial competition models, it produced the lowest estimates of growth rates for both *L. monocytogenes* and LAB in cheeses formulated with anti-listerial LAB, which were about half the estimates of the other two Jameson-effect variants. The interspecific competition models based on multi-species experiments could demonstrate a more accurate representation of microbial community dynamics in cheese and other food products. The developed interaction models for soft Minas cheese should prove valuable for future exposure and risk assessments to predict concentrations of *L. monocytogenes* and thereby assist in managing listeriosis risk.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fm.2018.11.004>.

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