This document contains the **post-print pdf-version** of the referred paper:

“Protective role of indigenous *Leuconostoc carnosum* against *Listeria monocytogenes* on vacuum packed Frankfurter sausages at suboptimal temperatures”

by Maria Baka, Estefanía Noriega, Laurence Mertens, Eva Van Derlinden, Jan F. Van Impe

which has been archived on the university repository Lirias (<https://lirias.kuleuven.be/>) of the Katholieke Universiteit Leuven.

The content is identical to the content of the published paper, but without the final typesetting by the publisher.

When referring to this work, please cite the full bibliographic info:

M. Baka, E. Noriega, L. Mertens, E. Van Derlinden J. F. Van Impe (2014). Protective role of indigenous Leuconostoc carnosum against Listeria monocytogenes on vacuum packed Frankfurter sausages at suboptimal temperatures. Food Research International, 66, 197-206.

The journal and the original published paper can be found at:

<https://www.sciencedirect.com/science/journal/09639969/66>
<https://www.sciencedirect.com/science/article/pii/S0963996914005493>

The corresponding author can be contacted for additional info.

Conditions for open access are available at:

<http://www.sherpa.ac.uk/romeo/>

Protective role of indigenous *Leuconostoc carnosum* against *Listeria monocytogenes* on vacuum packed Frankfurter sausages at suboptimal temperatures

Maria Baka^{a,b}, Estefanía Noriega^{a,b}, Laurence Mertens^{a,b},

Eva Van Derlinden^{a,b}, Jan Van Impe^{a,b,*}

^aCPMF2 – Flemish Cluster Predictive Microbiology in Foods – www.cpmf2.be

^bBioTeC – Chemical and Biochemical Process Technology and Control, Department of Chemical Engineering, KU Leuven, Leuven, Belgium.

[maria.baka, estefania.noriegafernandez, laurence.mertens, eva.vanderlinden, jan.vanimpe]
@cit.kuleuven.be.

***Author for correspondence:**

Jan F. M. Van Impe

BioTeC - Chemical and Biochemical Process Technology and Control

Department of Chemical Engineering, KU Leuven

Willem de Croylaan 46, B-3001. Leuven, Belgium

Tel: +32 16 32 14 66

Fax: +32 16 32 29 91

E-mail: jan.vanimpe@cit.kuleuven.be

ABSTRACT

Listeria monocytogenes is one of the main target pathogens in food industry. Novel strategies are continuously being investigated to ensure its absence from food products, such as the use of Lactic acid bacteria (LAB). They are known for their inhibitory action against pathogenic species due to the production of antimicrobial compounds and the competition for nutrients. In this work, the combined effect of storage temperature and LAB inoculum level on *L. monocytogenes* growth and the interaction between both microorganisms is examined on heat-treated Frankfurters. The indigenous LAB isolate from Frankfurter sausages was further identified as *Leuconostoc carnosum* and characterised as neither a bacteriocinogenic nor H₂O₂-producing species. However, it produces weak organic acids that acidify the food product and, overall, competes with *L. monocytogenes* for nutrients. Experiments were performed with vacuum packed, surface inoculated sausages, at different static temperatures (4, 8, 12 and 25°C) and inoculum levels of *L. carnosum* (10², 10³ and 10⁴ CFU/g) and 10² CFU/g *L. monocytogenes*. Results showed that at low temperature and high *L. carnosum* inoculum level, *L. monocytogenes* stops growing earlier than *L. carnosum* and the lowest maximum population is reached. The Dens et al. model described species interactions in a mechanistic way, revealing a predominant effect of *L. carnosum* on *L. monocytogenes*, and describing the decrease phase of *Listeria* population. The Baranyi and Roberts model, a special case of the Dens et al. model for monoculture, estimated apparent maximum population levels and brought into question the validity of the Jameson effect at low temperature. Results illustrated that indigenous species of meat products can be protective against foodborne pathogens.

Keywords: *Listeria monocytogenes*; LAB; *Leuconostoc carnosum*; Frankfurter sausages; inoculum level; temperature

1. Introduction

L. monocytogenes is one of the major threats in food industry, due to its virulence and ability to thrive even at very harsh conditions. This pathogen causes listeriosis, a severe invasive infection, which mainly affects unborn children, infants, the elderly and those with compromised immune systems. A total of 1642 confirmed cases of listeriosis were reported by 26 European Union Member States in 2012, with a high case fatality rate (17.8 %) among the cases. For these reasons, authorities have enforced strict regulations in order to ensure safe food products from *L. monocytogenes*. However, Ready-To-Eat (RTE) products, such as Frankfurter sausages, were among the categories with the highest levels of non-compliance to the regulations (2.5 %), at the processing level, in 2012 (EFSA/ECDC, 2014).

Lactic Acid Bacteria (LAB) are frequently used as bio-preservatives or protective cultures due to their natural ability to dominate the microflora of many foods where they naturally occur, e.g. meat products, which are chill-stored under vacuum or in an environment enriched with CO₂ (Holzapfel et al., 1995; Stiles, 1996; Mataragas et al., 2003). The competitive and frequently bacteriostatic effect of LAB has been investigated against pathogens such as *L. monocytogenes* and *Salmonella* spp. on foods.

Since the early sixties, the interaction of co-cultured species has been investigated. Jameson (1962) observed that if two species are co-cultured, there is one that dominates growth (predator) and the other that is suppressed (prey). This phenomenon was later suggested by Ross et al. (2000) to be called as Jameson effect. Many models have been implemented to describe autonomous, continuous-time, two species evolution, based on data from liquid microbiological media and real foods (Volterra, 1931; Lotka, 1956; Dubois et al., 1972; Coleman and Gomatam, 1972; Ayala et al., 1973; Schoener, 1976; McLean and Nowak, 1992; Dens et al., 1999; Vereecken et al., 2000). Additionally, several studies have been performed that prove this theory in liquid media (Buchanan and Klawitter, 1992; Foegeding et al., 1992;

Buchanan and Bagi, 1997; Schillinger et al., 1998; Buchanan and Bagi, 1999; Devlieghere et al., 2001; Pleasants et al., 2001; Szigeti, 2001; Greer and Dilts, 2006), in structured media (Antwi et al., 2007) and in food model systems (Winkowski and Montville, 1992; Jeppesen and Huss, 1993; Winkowski et al., 1993; Breidt and Fleming, 1998; Duffes et al., 1999; Palmai and Buchanan, 2002; Jacobsen et al., 2003; Nilsson et al., 2005; Vermeiren et al., 2006), although the simultaneous effect of the incubation temperature and the inoculum level of the co-cultured species has not been addressed yet. Nevertheless, this theory is a matter of discussion in the literature, since deviations have also been reported (Cornu, 2001; Mellefont et al., 2008; Mataragas et al., 2006; Le Marc et al., 2009).

In this study, the combined effect of storage temperature and inoculum level of an indigenous *L. carnosum* isolate on its co-culture with *L. monocytogenes* was assessed on Frankfurter sausages. In order to estimate growth parameters of co-cultured *L. carnosum* and *L. monocytogenes*, both bacterial growth curves were fitted with the Dens et al. model (1999), which explicitly takes species interactions into account and with the Baranyi and Roberts model (1994), a special case of the Dens et al. model, that fits the experimental data of each species independently of each other. In order to characterise the bacteriostatic / bactericidal effect of *L. carnosum* against *L. monocytogenes*, the evolution of sausage surface pH was monitored and standard assays unraveling the nature of a potential inhibitory effect were carried out.

2. Materials and methods

2.1. Bacterial strains

L. monocytogenes LMG 23775 strain, a sausage isolate, was acquired from the BCCM/LMG bacteria collection of Gent University in Belgium. A stock culture was stored at -80°C in Brain Heart Infusion broth (BHI, Oxoid), supplemented with 25% glycerol (v/v) (Acros Organics).

LAB were directly isolated in our laboratory from the Frankfurter sausages used for further analysis, by plating on de Man, Rogosa and Sharpe Agar (MRS, Oxoid). The purpose of using

Frankfurter isolates was to simulate realistic conditions. For the isolation, 25 g of Frankfurter sausages were mixed with 100 mL of sterile distilled water by stomaching (basic masticator, IUL INSTRUMENTS). 100 μ L of the suspension were poured on MRS agar plate and incubated for 48 h at 30°C. A selection of a few colonies formed in that plate were isolated by using a microbiological loop and added in sterile 10 mL MRS broth, which was incubated under the previously described conditions. This stationary phase cell suspension was used to prepare a stock culture, which was stored at -80°C in MRS broth (Oxoid), supplemented with 25% glycerol (v/v). Identification of LAB species was performed by 16S rRNA sequencing analysis method and they were found to belong to the *Leuconostoc carnosum* species. This analysis was performed in the KU Leuven Centre for Food and Microbial Technology.

2.2. Preculture conditions

For the preparation of the inoculum of *L. monocytogenes*, a loopful of the stock culture was inoculated into a 100 mL Erlenmeyer flask containing 20 mL BHI, which was incubated in a controlled incubator at 30°C (model KBP6151, Termaks). After 22 h, 20 μ L were transferred into 20 mL fresh BHI and again incubated at 30°C for 22 h.

For the preparation of the inoculum of *L. carnosum*, a loopful of the stock culture was inoculated into a test tube containing 10 mL MRS broth, and was incubated in a controlled incubator at 30°C for 48 h.

2.3. Characterisation of the inhibitory activity of *L. carnosum* against *L. monocytogenes*

In order to characterise the inhibitory activity of *L. carnosum* against *L. monocytogenes*, two assays were conducted. The first one, described by Harris et al. (1989), reveals if the inhibition of *Listeria* growth is induced by bacteriocins or weak organic acids produced by *L. carnosum*. In brief, 3 μ L of an overnight pre-culture of *L. carnosum* was spotted onto the surface of a BHI agar plate and incubated for 24 h at 30°C. Then, it was overlaid with 8 mL of BHI soft agar (0.75% w/v agar), seeded with 8 μ L of a *Listeria* preculture (10^9 CFU/mL). The plates were

incubated overnight at 37°C, and afterwards checked for inhibition zones, i.e., absence of growth of *Listeria* around *Leuconostoc* spots. Positive and negative controls were also included in the experimental design by using different agar media supporting *L. carnosum* growth: i) BHI-agar, where no acid inhibition should occur, due to the standard presence of 0.2% (w/v) glucose, ii) BHI-agar supplemented with 0.2% (w/v) glucose, to induce acid inhibition, and iii) BHI-agar supplemented with 1 mg/mL protease (P-5147, Sigma), to eliminate inhibition attributed to bacteriocins. Experiments were done in duplicate. The second assay, described by Amézquita and Brashears (2002), indicates if the inhibition of *L. monocytogenes* growth is due to a drop in pH, or bacteriocins and H₂O₂ production. *L. carnosum* was grown at 25°C for 48 h, under aerobic and vacuum conditions, in order to metabolise the potential inhibitory compounds. Cell free supernatants (CFS) were collected, as described in the original study, and the pH value was measured. *L. monocytogenes* was grown for 12 h at 37°C in i) MRS (control), ii) CFS, iii) CFS with pH adjusted to 6.5, to suppress the effect of weak organic acids, iv) CFS supplemented with 0.5 mg/mL catalase (C-40, Sigma), to suppress the effect from H₂O₂ and v) CFS supplemented with 1 mg/mL protease (P-5147, Sigma), to suppress the effect from bacteriocins. Experiments were conducted in duplicate. Evolution of *L. monocytogenes* concentration was monitored by sampling the different media, every 4 h.

2.4. Co-culture experiments in Frankfurters

Frankfurter sausages were purchased in a Belgian supermarket, in packs of 5 items (80 g / sausage). Immediately after their transfer to the laboratory, the sausages were maintained at 4°C before use. Prior to inoculation, sausages were dipped in boiling water for 30 s, in order to eliminate the background flora (Radin et al., 2006). Then, they were allowed to dry on sterile bandages and cool down under aseptic conditions for approximately 5 min. Early stationary phase cultures of *L. monocytogenes* and *L. carnosum* were mixed in the appropriate ratio in order to yield the target inoculation level of each species. Preliminary tests were performed in

order to select the most appropriate inoculation volume for the size / available surface of the sausages. The final volume was 60 μ L, in order to cover the maximum sausage surface and to minimise the drying period, before vacuum packaging. The inoculum levels of both *L. carnosum* and *L. monocytogenes* have been selected in order to represent realistic scenarios. Although the occurrence of *L. monocytogenes* is legally prohibited in ready-to-eat meat products, a limit concentration of 10^2 CFU/g is allowed for products at the consumption stage. This value has been used here to mimic the worst permitted scenario, as it also allows cell enumeration, with acceptable accuracy above the detection limit (>25 CFU / agar plate or 10^2 CFU / mL). *L. carnosum* inoculum densities were selected as 10^2 and 10^3 CFU/g, which are typical levels occurring in sausages and legally accepted for such a type of food product. An additional concentration of 10^4 CFU/g was also tested at 12 °C to address better the effect of the initial *L. carnosum* load at certain suboptimal temperature.

After inoculation, the cell solution was spread with a pipette tip over the entire sausage surface. Inoculated sausages were aseptically transferred into plastic bags of Polyamide / Polyethylene (PA/PE 90), and left in at 4°C for 15 min, to allow cell attachment (Byelashov et al., 2008). Finally, they were packaged under 99% vacuum in a vacuum machine (Boxer 42, Henkelman) and incubated at four different temperatures (4, 8, 12 and 25°C) for maximum 30 days. These temperatures were selected based on the normal storage temperature for this type of meat products (4°C), abuse temperatures (8 and 12°C) and ambient temperature (25°C) for extensive mishandling of the products during summer time. All samples were vacuum packed prior to incubation (one sausage per bag).

2.5. Microbiological analysis in Frankfurters

Evolution of *L. monocytogenes* and *L. carnosum* concentrations over time was monitored by viable plate counting. Standard volume (240 mL) of peptone water (10 g/L bacteriological peptone (Oxoid) and 5 g/L NaCl (AnalaR, Normapur®, VWR International)) was aseptically

poured in the vacuum bag, and mixed with the whole sausage for 2 min by stomaching. Then, the corresponding serial decimal dilutions were plated with a Spiral Plate System (Eddy Jet IUL Instruments), on Palcam agar base with Palcam selective supplement (Oxoid) and on MRS agar (Oxoid), for the enumeration of *L. monocytogenes* and *L. carnosum*, respectively. It is worth mentioning that close to the inoculation time, for inoculum levels of 10^2 CFU/g, instead of plating 49.2 μ L with the Spiral Plate System, 100-1000 μ L of sample was spread in order to permit countable plates (>25 CFU / agar plate). Plates were incubated at 30°C for 48 h before enumeration. The sampling frequency until growth curves reached the stationary phase was 1, 2, 3 and 15 samples / day at 4, 8, 12 and 25°C, respectively. Afterwards, sampling time frequency was reduced up to 1 to 2 samples / day. It is noteworthy that a number of sausages (between 20 and 30) were inoculated simultaneously at the beginning of each experiment and one sausage was sampled at each time interval. Every experiment was independently performed in duplicate.

2.6. pH measurement

At specific time intervals, the pH was measured on the surface of the sausage at three different points and the average value was calculated. The pH electrode (HI 1413B, Hanna Instruments) was appropriate for measuring the pH on food surfaces.

2.7. Modelling

The Dens et al. model, described by equations (1), was applied in its implicit form to co-cultured *L. monocytogenes* (subscript '1') and *L. carnosum* (subscript '2'), in order to describe potential species interactions.

$$\begin{aligned}\frac{dN_1(t)}{dt} &= \frac{Q_1(t)}{1+Q_1(t)} \cdot \mu_{\max 1} \cdot \left(\frac{N_1(t)}{N_{\max 1}} \right) \cdot (N_{\max 1} - N_1(t) - a_{12} \cdot N_2(t)) \\ \frac{dN_2(t)}{dt} &= \frac{Q_2(t)}{1+Q_2(t)} \cdot \mu_{\max 2} \cdot \left(\frac{N_2(t)}{N_{\max 2}} \right) \cdot (N_{\max 2} - N_2(t) - a_{21} \cdot N_1(t))\end{aligned}\quad (1)$$

$$\frac{dQ_1(t)}{dt} = \mu_{\max 1} \cdot Q_1(t)$$

$$\frac{dQ_2(t)}{dt} = \mu_{\max 2} \cdot Q_2(t)$$

With $N_{1,2}(t)$ [CFU/g], the cell density of both species at time t ; $N_{\max 1,2}$ [CFU/g], the intrinsic maximum value for $N(t)$ of both species (i.e., when no other species is present); $Q_{1,2}(t)$ [-], the physiological state of the cells of both species; $\mu_{\max 1,2}$ [1/h], the maximum specific growth rate of both species; a_{12} [-] and a_{21} [-], the interaction coefficients measuring the effects of species 2 to species 1 and of species 1 to species 2, respectively.

When equations (1) are solved for only one species, then the Dens et al. model becomes the Baranyi and Roberts model, described by equations (2), which does not consider species interactions. The Baranyi and Roberts model was fitted, in its implicit form, to the experimental data of *L. monocytogenes* mono-culture and to the data where *L. monocytogenes* was in co-culture with *L. carnosum*, in order to compare it with the fit of the Dens et al. model.

$$\begin{aligned}\frac{dN(t)}{dt} &= \frac{Q(t)}{1+Q(t)} \cdot \mu_{\max} \cdot \left(1 - \frac{N(t)}{N_{\max -ap}} \right) \cdot N(t) \\ \frac{dQ(t)}{dt} &= \mu_{\max} \cdot Q(t)\end{aligned}\quad (2)$$

With $N(t)$ [CFU/g], the cell density at time t ; $N_{\max -ap}$ [CFU/g], the apparent maximum value for $N(t)$, when the Baranyi and Roberts model is fitted independently to each species in co-culture; $Q(t)$ [-], the physiological state of the cells; and $\mu_{\max}$ [1/h], the maximum specific growth rate.

The time instant for the onset of the stationary phase of each growth curve was estimated as the intersection time at which the exponential phase meets the upper asymptote of the stationary phase by using the estimated parameters (lag, $\mu_{\max}$, $N_{\max-ap}$) of the Baranyi and Roberts model for each experimental condition. Furthermore, the difference between the onset time of *L. monocytogenes* ($t_{L.monoc}$) and *L. carnosum* ($t_{L.carn}$) was expressed as a percentage of the onset time of *L. carnosum* ((%) $t_{L.monoc-decrease}$), as described by equation (3).

$$(\%)t_{L.monoc-decrease} = \frac{t_{L.carn} - t_{L.monoc}}{t_{L.carn}} \cdot 100\% \quad (3)$$

2.8. Parameter estimation

Model parameters were estimated from the set of experimental data via the minimisation of the sum of squared errors, using the lsqnonlin routine of the Optimization Toolbox of Matlab version R2010b (The Mathworks Inc.). For parameter estimation based on one experiment (with M measurements), the Mean Squared Error (MSE) is described by equation (4).

$$SSE = \sum_{i=1}^M (n(t_i) - n(t_i, \mathbf{p}))^2 \quad (4)$$

$$MSE = \frac{SSE}{M - \nu}$$

With SSE the Sum of Squared Errors, $n(t_i)$ the natural logarithm of the cell density by measurements and $n(t_i, \mathbf{p})$ the natural logarithm of the cell density by the model prediction at time t_i . The vector $\mathbf{p}$ contains the considered parameters of the model. ν stands for the number of degrees of freedom.

2.9. Statistical analysis

Analysis of variance (ANOVA) test was performed to determine whether there are any significant differences amongst means of parameter estimates, at a 95.0% confidence level ($\alpha = 0.05$). Fisher's Least Significant Difference (LSD) test was used to distinguish which means were significantly different from which others. Standardized skewness and standardized kurtosis were used to assess if data sets came from normal distributions. These analyses were

performed using Statgraphics Centurion XVI.I® Package (Statistical Graphics, Washington, USA). Test statistics were regarded as significant when P was ≤ 0.05 .

3. Results

3.1. Characterisation of antilisterial activity of *L. carnosum*

In Figure 3, the positive or negative occurrence of inhibition zones of *L. monocytogenes* around spots of *L. carnosum* is depicted on: (3a) BHI-agar, (3b) BHI-agar supplemented with 0.2% (w/v) glucose and (3c) BHI-agar supplemented with 1 mg/mL protease. No inhibition zone is observed in the cases of normal BHI-agar and BHI-agar supplemented with protease. However, when BHI is supplemented with 0.2% (w/v) glucose, *Listeria* growth is suppressed in the proximity of *Leuconostoc* spots and then, slight inhibition zones are formed (thin black halo around spots). Therefore, this assay illustrates that the selected *L. carnosum* strain is not bacteriocinogenic, but inhibits *Listeria* growth by the production of weak organic acids and the subsequent drop in pH. In Figure 4, results of the second assay are depicted. Thereto, *L. monocytogenes* grows in the control (MRS) and the CFS with pH adjusted to 6.5. In contrast, *Listeria* almost remained equal to the initial inoculum level in the CFS, the CFS supplemented with catalase and protease. These results are in accordance with the conclusions of the previous assay, i.e., the inhibition of *Listeria* is not due to bacteriocins or H_2O_2 production, but mainly due to the production of weak organic acids resulting in a pH drop.

3.2. Combined effect of temperature and *L. carnosum* inoculum level on growth parameters of *L. monocytogenes*

Figure 1 illustrates the influence of temperature and *L. carnosum* inoculum level on *L. monocytogenes* growth dynamics, as well as the corresponding growth curves of *L. carnosum*. The Dens et al. model fit to experimental data of *L. monocytogenes* and *L. carnosum* is presented with red and blue continuous lines, respectively. The Baranyi and Roberts model fit, which is a special case of the Dens et al. model without considering species interactions, is

depicted with black and fuchsia dashed lines, respectively. Growth curves of mono-cultured *L. monocytogenes* were fitted with the Baranyi and Roberts model (Figure 2). The growth parameters of both microorganisms estimated by the Dens et al. and the Baranyi and Roberts models are summarised in Tables 1 and 2, respectively.

In table 1, the effect of *L. carnosum* on *L. monocytogenes* and vice versa is quantitatively described by the interaction coefficients of the Dens et al. model (a_{12} and a_{21}). During a preliminary parameter estimation, the values of a_{21} (describing the effect of *L. monocytogenes* on *L. carnosum*) were found to be negligibly small while exhibiting unrealistically large confidence intervals. Biologically speaking, this means that the effect of *L. monocytogenes* on *L. carnosum* can be neglected. So, the parameter a_{21} was accordingly set equal to zero during final parameter estimation. Similarly, preliminary results pointed out that, in quite some cases, the estimated values for the lag duration approached the biologically lower bound of zero, with unrealistically large confidence intervals. In these cases, lag duration was set equal to 0 hours, also in agreement with the experimental data (see Figure 1.1 and 1.2). In the rest of the cases, lag phase could not be neglected, as the estimates were significant values directly detectable at the growth curves. In all these cases, the standard error of the estimate is relatively high, but this is due to the lack of adequate points at this part of the growth curve necessary for accurate estimation of the lag parameter. For *L. monocytogenes*, the $N_{\max 1}$ value in the Dens et al. model (Table 1), which represents the intrinsic maximum population without species interaction, was set equal to its maximum mono-culture value (Table 2). Mono-culture data of *L. carnosum* were not available under the experimental conditions of this study, so the intrinsic maximum population density $N_{\max,2}$ was not fixed, but estimated. For the specific set of conditions 4°C - 10³ CFU/g *L. carnosum*, the maximum specific growth rate from the Baranyi and Roberts model was set to 0 (1/h), since no significant microbial growth was experimentally observed.

The growth of *L. monocytogenes* was significantly suppressed by the presence of *L. carnosum* at all temperatures (Figure 1.1 and 1.2), since the intrinsic $N_{\max,1}$ as a mono-culture obtained by the Baranyi and Roberts model, as a special case of the Dens et al. model was never reached (Table 2). A very short exponential phase is also observed, as compared to its mono-culture, which is illustrated in Figure 2. Moreover, when *L. carnosum* inoculum level is 10^3 CFU/g, the lower the temperature, the earlier *L. monocytogenes* enters the stationary phase in comparison to *L. carnosum*, i.e., the (%) $t_{L.\text{monoc-decrease}}$, in Table 3, is higher at low temperatures. When the inoculum level of *L. carnosum* is 10^2 CFU/g, this time value remains approximately at the same level, apart from the case of 25°C, at which it is significantly lower (Table 3). In the case where the inoculum level of *L. carnosum* is 10^4 CFU/g, the $t_{L.\text{monoc-decrease}}$ is negative, due to the very limited growth of *L. monocytogenes*. These results are an indication of the effect of temperature and *L. carnosum* inoculum level on the validity of the Jameson effect. Additionally, it is observed that the higher the initial load of *L. carnosum*, the earlier *L. monocytogenes* enters its stationary phase with respect to the time required by *L. carnosum*, at 4 and 8°C. On the other hand, at 12 and 25°C *L. monocytogenes* enters the stationary phase faster at lower inoculum levels (Table 3). Additionally, it is obvious from Figure 1 and the apparent $N_{\max-\text{ap}}$ values listed in Table 2 that *L. monocytogenes* reaches higher maximum population densities when co-cultured with lower inoculum of *L. carnosum* at higher temperatures. The combination of the lowest temperature and the highest inoculum level of *L. carnosum* lead to the highest time difference between *L. monocytogenes* and *L. carnosum* entering their stationary phase. These two factors act additively for the suppression of *L. monocytogenes* growth. According to the Dens et al. model fit, *L. monocytogenes* population started decreasing with time after reaching its maximum population, decrease phase that was not described by the Baranyi and Roberts model.

3.3. Effect of pH on growth dynamics of *L. monocytogenes*

In Figure 2, the evolution of pH is represented as mono-cultured *L. monocytogenes* concentration is given with time. The observed drop of pH is due to the products of *Listeria* metabolism (Romick et al., 1996). As depicted in Figure 1, *L. monocytogenes* stops growing while pH is at the same level as the pH value of sausages at the moment of inoculation. Exceptionally, at 25°C *L. monocytogenes* enters the stationary phase when pH has dropped to 5.5 from 5.8, which is close to the initial value found at the Frankfurters.

4. Discussion

In this study, the Dens et al. model and the special case without considering species interactions, the Baranyi and Roberts models were used to estimate the growth parameters of co-cultured *L. monocytogenes* and *L. carnosum*. The Dens et al. model provides quantitative estimates of species interaction, i.e., the interaction coefficients. In addition, this model describes *Listeria* population decrease phase, induced by the presence of *L. carnosum*. This is valuable information obtained by the fit of the Dens et al. model, as this type of food products have a shelf-life of 7-10 days (170-240h), where in most cases the deceleration of *L. monocytogenes* occurs. The Baranyi and Roberts model describes independently both bacterial growth curves, without considering species interaction. So, this model estimates the apparent maximum population reached by both microorganisms in co-culture, which is influenced by species interaction and underestimated due to the decrease phase of *L. monocytogenes*. However, the apparent $N_{\max\text{-ap}}$ provides an estimation of *Listeria* growth suppression, as it is lower than the $N_{\max1}$ in mono-culture. The time instant at which the stationary phase starts, is calculated by using the estimated growth parameters of the Baranyi and Roberts model. This is computed as the intersection time at which the exponential phase meets the upper asymptote of the stationary phase. By comparing the (%) $t_{L.\text{monoc-decrease}}$, the validity of the Jameson effect is brought into question. As the apparent $N_{\max\text{-ap}}$, estimated by the Baranyi and Roberts model, is involved for the computation of the onset time of the stationary phase, this time value is

influenced by the above mentioned uncertainty in $N_{\max-ap}$ estimation. Therefore, shorter onset time of the stationary phase for *L. monocytogenes* is estimated, when the decrease phase is present (8 and 12°C). It is noteworthy that the Dens et al. model fits better the data throughout the storage time than the Baranyi and Roberts model, as it describes the growth and the decrease phase of *L. monocytogenes*.

Jameson (1962) originally observed that when two species are co-cultured in liquid microbiological media, they are distinguished as predators and preys, as explained before. Specifically, when the dominant microorganism enters the stationary phase, the prey subsequently follows it, although it has not reached its potential final population. Dominant microorganisms are normally those that grow faster as mono-culture under the same conditions. This theory is based on the co-culture of *Salmonella litchfield* and *Escherichia coli* at 37, 41 and 44°C. In the present study, according to the experimental data at 25°C (Figure 1.2 h and i) Jameson effect is followed, independently of the inoculum level of *L. carnosum*. Additionally, experimental data at 12°C (Figure 1.2 e, f and g) tend to follow this theory, especially when inoculum of *L. carnosum* increases. However, data at 4°C and 8°C (Figures 1.1 a, b, c, and d, Table 3) deviate from Jameson's theory. Consequently, the lower the storage temperature (less optimal temperature), the more the results deviate this effect. This observation suggests a significant effect of incubation / storage temperature on the interaction of both species. An explanation for this may be the hurdle theory (Leistner et al., 1995). If low temperature and the depletion of / competition for nutrients are considered as hurdles, then, *L. monocytogenes* growth can be suppressed much earlier at lower temperatures. The results here obtained can give an explanation to the studies reporting non-compliance with Jameson's approach (Mataragas et al., 2006; Le Marc et al., 2009 and Cornu et al., 2001). Thereto, data were taken at [4, 8, 12, 16°C] in the first case, and the deviation from the Jameson effect was more evident at 4 and 8°C. In the second case, data were taken at [18 and 25°C] and in the third study at 8°C.

These temperatures are below the optimal for the species studied, i.e., *Staphylococcus aureus* (second case) and *L. monocytogenes* (first and third case). In contrast, in Jameson's study, incubation temperatures were close to the optimal for growth of both species.

The underlying mechanisms supporting the Jameson's theory are complex and may be explained by a combination of effects, such as production of antimicrobials and competition for / or depletion of specific nutrients (Devlieghere et al., 2004). Though, the major reason of bacterial growth suppression between co-cultured species is reported to be the competition for nutrients. This is considered to be one of the major evolutionary driving forces in the bacterial world, and numerous experimental data show how different organisms, or variants of one organism, may effectively outcompete others because of a better utilization of a given energy source (Christensen et al., 2002). Alternatively, the inhibition of the prey's growth may be due to the production of bacteriocins from the predator, which are peptides with antimicrobial activity. However, not all "predator" microorganisms produce bacteriocins (Buchanan and Bagi, 1997; Nilsson et al., 1999; Nilsson et al., 2004; Nilsson et al., 2005). Moreover, organic acids or hydrogen peroxide, produced by the dominant species, are found to have a bacteriostatic effect (Barakat and Harris, 1999; Nilsson et al., 1999; Amezcuita and Brashears, 2002). If none of the two cases occur, it is evident that the two species compete for nutrients.

LAB, including *L. carnosum*, are known to produce lactic acid that acidifies the surrounding environment (Juven et al., 1998) and some species belonging to this group can also be bacteriocinogenic. In this study, both assays, conducted to characterise the antilisterial activity of *L. carnosum*, revealed that the inhibition is not attributed to bacteriocins or H₂O₂ production, but most likely due to the production of weak organic acids resulting in a pH drop. Additionally, it is observed from the pH measurements that *Listeria* growth is suppressed while pH still remains at the initial value of the sausages, independently of the tested conditions (Figure 1). By combining the latter observations, it can be concluded that *Listeria* growth is initially

suppressed due to nutrients competition between the two species and furthermore, by the production of weak organic acids resulting in a pH drop.

L. carnosum, as the dominant species of LAB isolated from Frankfurters, has a bacteriostatic and even bactericidal with time effect against *Listeria*, enhanced by the low incubation temperature and the potential competition for nutrients between the two species.

In several studies, the effect of the inoculum size on the inhibition against competitors has been reported (Buchanan and Klawitter, 1992; Devlieghere et al., 2001; Szigeti, 2001; Nilsson et al., 2005; Vermeiren et al., 2006). Mellefont et al. (2008) have described the importance of the initial inoculum of the two species to define the role of prey-predator. Thereto, if one species has higher initial load, then it dominates growth. Additionally, Malakar et al. (2003) suggested that the inhibitory effect of the competitive microflora on pathogens only occurs when the population of the competitive microflora reaches 10^8 CFU/mL. However, in this study, the time at which the final population of *L. monocytogenes* is reached does not depend on the population level reached by *L. carnosum*, as shown in Figure 1. Moreover, when the initial load of *L. carnosum* is equal to that of *L. monocytogenes* (10^2 CFU/g), *L. carnosum* still remain the dominant species. This was quantitatively confirmed by the interaction coefficients of the Dens model et al. (Table 1), as discussed above. This bacteriostatic / bactericidal effect of *L. carnosum* at low initial loads, which are below the maximum acceptable limit for RTE products consumption ($<10^7$ CFU/g) (ICMSF, 1986), is important to prevent *Listeria* growth in cold-stored, vacuum-packed Frankfurters. $N_{\max\text{-ap}}$ values of *Listeria* in Table 2 and a_{12} values in Table 1 show that the inoculum level of *L. carnosum* influences the final population of *L. monocytogenes*. Specifically, when the initial inoculum of *L. carnosum* is higher, *Listeria* reaches lower apparent maximum population ($N_{\max\text{-ap}}$) and stops growing earlier in comparison to data where *L. carnosum* had lower initial load (Table 3). Additionally, under the same condition, the time difference of *Listeria* and *L. carnosum* to enter the stationary phase is higher

(Table 3). In conclusion, the results here presented suggest a multifactorial antimicrobial effect dependent on the storage temperature, *L. carnosum* inoculum level, the production of weak organic acids by *L. carnosum*, and / or the depletion of nutrients.

5. Conclusions

This research demonstrates that the combined effect of storage temperature and inoculum level of *L. carnosum* play an important role on the growth suppression of *L. monocytogenes* when the two species are co-cultured. Specifically, at the lowest storage temperature and the highest inoculum level of *L. carnosum*, the growth of *Listeria* is the most limited with respect to $N_{\max-ap}$ and the time required to enter the stationary phase. At normal refrigeration temperature for RTE products (4°C), *Listeria* stops growing earlier in comparison to *L. carnosum*, than at abuse temperatures (8, 12°C) or at ambient temperature, when co-cultured with *L. carnosum* (the dominant flora). The bacteriostatic and subsequent bactericidal effect of *L. carnosum* against *Listeria* was pronounced even when both bacteria have equal and very low initial load (10^2 CFU/g). The bacteriostatic / bactericidal effect of *L. carnosum* on *L. monocytogenes* was attributed to their competition for nutrients and the production of weak organic acids resulting in a pH drop. Regarding growth parameter estimation, the Dens et al. model confirmed numerically a significant effect of *L. carnosum* on *L. monocytogenes* and described the *Listeria* population decrease phase, after reaching its maximum population and the Baranyi and Roberts model fit brought into question the validity of the Jameson effect at low temperatures. The information presented in this work contributes to a better understanding of the influence of environmental conditions, specifically temperature, on the interaction between both species and highlights the potential protective role of the background microflora of food products against foodborne pathogens.

Acknowledgements

This work was supported by project PFV/10/002 (Center of Excellence OPTEC-Optimization in Engineering) of the KU Leuven Research Fund, Knowledge Platform KP/09/005 (www.scores4chem.be) of the KU Leuven Industrial Research Fund, project G.0930.13 of the Fund for Scientific Research-Flanders, and the Belgian Program on Interuniversity Poles of Attraction, initiated by the Belgian Federal Science Policy Office (IAP Phase VII - 19 DYSCO). Professor Jan Van Impe holds the chair Safety Engineering sponsored by the Belgian chemistry and life sciences federation essenscia. Finally, we would like to thank Professor Abram Aertsen and the laboratory of Centre for Food and Microbial Technology, at the Faculty of Bioscience Engineering, KU Leuven, for their support in the identification of the LAB.

References

- Amézquita, A., Brashears, M.M., 2002. Competitive inhibition of *Listeria monocytogenes* in ready-to-eat meat products by lactic acid bacteria. *Journal of Food Protection* 65, 315-325.
- Antwi, M., Bernaerts, K., Van Impe, J.F., Geeraerd, A.H., 2007. Modelling the combined effects of structured food model system and lactic acid on *Listeria innocua* and *Lactococcus lactis* growth in mono- and co-culture. *International Journal of Food Microbiology* 120, 71-84.
- Ayala, F.J., Giplin, M.J., Ehrenfield, J.G., 1973. Competition between species: theoretical models and experimental results. *Theoretical Population Biology* 4, 331-356.
- Barakat, R.K., Harris, L.J., 1999. Growth of *Listeria monocytogenes* and *Yersinia enterocolitica* on cooked modified-atmosphere-packaged poultry in the presence and absence of a naturally occurring microbiota. *Applied Environmental Microbiology* 65, 342-345.
- Baranyi, J., Roberts, T.A., 1994. A dynamic approach to predicting bacterial growth in food. *International Journal of Food Microbiology* 23, 277-294.

- Breidt, F., Fleming, H.P., 1998. Modelling of the competitive growth of *Listeria monocytogenes* and *Lactococcus lactis* in vegetable broth. *Applied and Environmental Microbiology* 64, 3159–3165.
- Buchanan, R.L., Bagi, L.K., 1997. Microbial competition: effect of culture conditions on the suppression of *Listeria monocytogenes* Scott A by *Carnobacterium piscicola*. *Journal of Food Protection* 60, 254–261.
- Buchanan, R.L., Bagi, L.K., 1999. Microbial competition: effect of *Pseudomonas fluorescens* on the growth of *Listeria monocytogenes*. *Food Microbiology* 16, 523–529.
- Buchanan, R.L., Klawitter, L.A., 1992. Characterization of lactic acid bacterium, *Carnobacterium piscicola* LK5, with activity against *Listeria monocytogenes* at refrigerated temperatures. *Journal of Food Safety* 12, 199–217.
- Byelashov, O.A., Simpson, C.A., Geornaras, I., Kendall, P.A., Scanga, J. A., Sofos, J. N., 2008. Evaluation of changes in *Listeria monocytogenes* populations on frankfurters at different stages from manufacturing to consumption. *Food Microbiology and Safety* 73, 430-437.
- Christensen, B.B., Haagensen, J.A., Heydorn, A. And Molin, S., 2002. Metabolic commercialism and competition in a two-species microbial consortium. *Applied and Environmental Microbiology* 68, 2495-2502.
- Coleman, T.P., Gomatam, J., 1972. Application of a new model of species competition to *Drosophila*. *National New Biology* 239, 251-253.
- Cornu, M., 2001. Modelling the competitive growth of *Listeria monocytogenes* and food flora in situ. *Acta Horticultura* 566, 151-157.
- Dens, E.J., Vereecken, K.M., Van Impe, J.F., 1999. A prototype model structure for mixed microbial populations in homogeneous food products. *Journal of Theoretical Biology* 201, 159-170.

- Devlieghere, F., Geeraerd, A.H., Versyck, K.J., Vandeaeataere, B., Van Impe, J.F., Debevere, J., 2001. Growth of *Listeria monocytogenes* in modified atmosphere packed cooked meat products: a predictive model. Food Microbiology 18, 53–66.
- Devlieghere, F., Vermeiren, L., Debevere, J., 2004. New preservation technologies: possibilities and limitations. International Dairy Journal 14 (4), 273–285.
- Dubois, D.M., 1978. State-of-the-art in predator-prey systems modelling. In: State-of-the-Art in Ecological Modelling (Jorgensen, S. E., ed.), Proceedings of the ISEM Conference on ecological modelling, Copenhagen, Denmark, 28 August-2 September 1978, pp. 163-217.
- Duffes, F., Corre, C., Leroi, F., Dousset, X., Boyaval, P., 1999. Inhibition of *Listeria monocytogenes* by in situ produced and semi-purified bacteriocins of *Carnobacterium* spp. on vacuum-packed, refrigerated cold-smoked salmon. Journal of Food Protection 62, 1394–1403.
- EFSA/ECDC., 2014. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2012. EFSA Journal 2014, 12 (2):3547.
- Foegeding, P.M., Thomas, A.B., Pilkington, D.H., Klaenhammer, T.R., 1992. Enhanced control of *Listeria monocytogenes* by insitu-produced pediocin during dry fermented sausage production. Applied and Environmental Microbiology 58, 884–890.
- Greer, G.G., Dilts, B.D., 2006. Control of meat-borne *Listeria monocytogenes* and *Brochothrix thermosphacta* by a bacteriocinogenic *Brochothrix campestris* ATCC 43754. Food Microbiology 23, 785–790.
- Harris, L.J., Daeschel, M.A., Stiles, M.E., Klaenhammer, T.R., 1989. Antimicrobial activity of Lactic Acid Bacteria against *Listeria monocytogenes*. Journal of Food Protection 52, 384-387.

- Holzappel, W.H., Geisen, R., Schillinger, U., 1995. Biological preservation of foods with reference to protective cultures, bacteriocins and food grade enzymes. *International Journal of Food Microbiology* 24, 343–362.
- ICMSF., 1986. *Microorganisms in Foods 2, Sampling for Microbiological Analysis: Principles and Specific Applications*. (2nd Ed.) International Commission on Microbiological Specifications for Foods, *Toronto: University of Toronto Press*.
- Jacobsen, T., Budde, B.B., Koch, A.G., 2003. Application of *Leuconostoc carnosum* for bio-preservation of cooked meat products. *Journal of Applied Microbiology* 95, 242–249.
- Jameson, J., 1962. A discussion of the dynamics of *Salmonella* enrichment. *Journal of Hygiene* 60, 193-207.
- Jeppesen, V.F., Huss, H.H., 1993. Antagonistic activity of 2 strains of lactic acid bacteria against *Listeria monocytogenes* and *Yersinia enterocolitica* in a model fish product at 5°C. *International Journal of Food Microbiology* 19, 179–186.
- Juven, B.J., Barefoot, S.F., Pierson, M.D., McCaskill, L.H., Smith, B., 1998. Growth and survival of *Listeria monocytogenes* in vacuum-packaged ground beef inoculated with *Lactobacillus alimentarius* FloraCarn L-2. *Journal of Food Protection* 61, 551– 556.
- Le Marc Y., Valík L., Medvedová A., 2009. Modelling the effect of the starter culture on the growth of *Staphylococcus aureus* in milk. *International Journal of Food Microbiology* 129, 306-311.
- Leistner, L. (1995). Principles and applications of hurdle technology. In G. W. Gould (Ed.), *New methods for food preservation* (pp. 1-21). London: Blackie Academic & Professional.
- Lotka, A. J., 1956. *Elements of mathematical biology*. New York: Dover.
- Malakar, P.K., Barker, G.C., Zwietering, M.H., van't Riet, K., 2003. Relevance of microbial interactions to predictive microbiology. *International Journal of Food Microbiology* 84, 263-272.

- Mataragas, M., Drosinos, E.H., Metaxopoulos, J., 2003. Antagonistic activity of lactic acid bacteria against *Listeria monocytogenes* in sliced cooked cured pork shoulder stored under vacuum or modified atmosphere at 4±2°C. Food Microbiology 20, 259-265.
- Mataragas, M., Drosinos, E.H., Siana, P., Skandamis, P., Metaxopoulos, I., 2006. Determination of the growth limits and kinetic behavior of *Listeria monocytogenes* in a sliced cooked cured meat product: Validation of the predictive growth model under constant and dynamic temperature storage conditions. Journal of Food Protection 69(6), 1312-1321.
- Mellefont L.A., McMeekin T.A., Ross T., 2008. Effect of relative inoculum concentration on *Listeria monocytogenes* growth in co-culture. International Journal of Food Microbiology 121, 157-168.
- McLean, A.R., Nowak, M.A., 1992. Models of interactions between HIV and other pathogens. Journal of Theoretical Biology 155, 69-102.
- Nilsson, L., Gram, L., Huss, H., 1999. Growth control of *Listeria monocytogenes* on cold-smoked salmon using competitive lactic acid bacteria flora. Journal of Food Protection 62, 336–342.
- Nilsson, L., Ng, Y.Y., Christiansen, J.N., Jorgensen, B.L., Grotnum, D., Gram, L., 2004. The contribution of bacteriocin to inhibition of *Listeria monocytogenes* by *Carnobacterium piscicola* strains in cold-smoked salmon systems. Journal of Applied Microbiology 96, 133–143.
- Nilsson, L., Hansen, T.B., Garrido, P., Buchrieser, C., Glaser, P., Knochel, S., Gram, L., Gravesen, A., 2005. Growth inhibition of *Listeria monocytogenes* by a non-bacteriocinogenic *Carnobacterium piscicola*. Journal of Applied Microbiology 98, 172–183.
- Palmai, M., Buchanan, R.L., 2002. The effect of *Lactococcus lactis* on the growth characteristics of *Listeria monocytogenes* in alfalfa sprout broth. Acta Alimentaria 31, 379–392.

- Pleasants, A.B., Soboleva, T.K., Dykes, G.A., Jones, R.J., Filippov, A.E., 2001. Modelling of the growth of populations of *Listeria monocytogenes* and a bacteriocin-producing strain of *Lactobacillus* in pure and mixed cultures. Food Microbiology 18, 605–615.
- Radin, D., Niebuhr, S.E., Dickson, J.S., 2006. Impact of the population of spoilage microflora on the growth of *Listeria monocytogenes* on frankfurters. Journal of Food Protection 69, 679-681.
- Romick, T.L., Fleming, H. P., McFeeters, R.F., 1996. Aerobic and anaerobic metabolism of *Listeria monocytogenes* in defined glucose medium. Applied and Environmental Microbiology 62 (1), 604-307.
- Ross T., Dalgaard P., Tienungoon S., 2000. Predictive modelling of the growth and survival of *Listeria* in fishery products. International Journal of Food Microbiology 62, 231-245.
- Schoener, T.W., 1976. Alternatives to Lotka-Volterra competition: models of intermediate complexity. Theoretical Population Biology 10, 309-333.
- Stiles, M.E., 1996. Bio-preservation by lactic acid bacteria. Antonie Van Leeuwenhoek 70, 331–345.
- Schillinger, U., Chung, H.S., Keppler, K., Holzapfel, W.H., 1998. Use of bacteriocinogenic lactic acid bacteria to inhibit spontaneous nisin-resistant mutants of *Listeria monocytogenes* Scott A. Journal of Applied Microbiology 85, 657–663.
- Szigeti, E., 2001. Interaction between *Lactococcus lactis* strains and *Listeria monocytogenes* in mixed cultures. Acta Alimentaria 30, 247–265.
- Vereecken, K.M., Dens, E.J., Van Impe, J.F., 2000. Predictive modelling of mixed microbial populations in food products: Evaluation of two-species Models. Journal of Theoretical Biology 205, 53-72.
- Vermeiren, L., Devlieghere, F., Vandekinderen, I., Debevere, J., 2006. The interaction of the non-bacteriocinogenic *Lactobacillus sakei* 10A and lactocin S producing *Lactobacillus sakei*

148 towards *Listeria monocytogenes* on a model cooked ham. Food Microbiology 23, 511–518.

Volterra, V., 1931. Leçons sur la théorie mathématique de la lutte pour la vie. Paris: Goutliers-Vallard.

Winkowski, K., Montville, T.J., 1992. Use of meat isolate, *Lactobacillus bavaricus* MN, to inhibit *Listeria monocytogenes* growth in a model meat gravy system. Journal of Food Safety 13, 19–31.

Winkowski, K., Crandall, A.D., Montville, T.J., 1993. Inhibition of *Listeria monocytogenes* by *Lactobacillus bavaricus* MN in beef systems at refrigeration temperatures. Applied and Environmental Microbiology 59, 2552–2557.

Figure Captions

Figure 1.1. Viable counts of co-cultured *L. monocytogenes* (●) and *L. carnosum* (▲) at 4°C (a, b) and at 8°C (c, d). The inoculum level of *L. carnosum* is 10² CFU/g (a, c) and 10³ CFU/g (b, d). The Baranyi and Roberts model fit is indicated by dashed lines: (--) for *L. monocytogenes* and (--) for *L. carnosum*. The Dens et al. model fit is indicated by continuous lines: (-) for *L. monocytogenes* and (-) for *L. carnosum*. pH evolution is depicted by solid symbols (●).

Figure 1.2. Viable counts of co-cultured *L. monocytogenes* (●) and *L. carnosum* (▲) at 12°C (e, f, g) and 25°C (h, i). The inoculum level of *L. carnosum* is 10² CFU/g (e, h), 10³ CFU/g (f, i) and 10⁴ CFU/g (g). The Baranyi and Roberts model fit is indicated by dashed lines: (--) for *L. monocytogenes* and (--) for *L. carnosum*. The Dens et al. model fit is indicated by continuous lines: (-) for *L. monocytogenes* and (-) for *L. carnosum*. pH evolution is depicted by solid symbols (●).

Figure 2. Viable counts of mono-cultured *L. monocytogenes* (●) at 4 (a), 8 (b), 12 (c) and 25°C (d). The Baranyi and Roberts model fit is indicated by the dashed line (--) and pH evolution, by solid symbols (●).

Figure 3. Positive or negative inhibition zones of *L. monocytogenes* around *L. carnosum*, spots on (a) BHI-agar, (b) BHI-agar supplemented with 0.2% glucose and (c) BHI-agar supplemented with 1 mg/mL protease.

Figure 4. Viable counts ($\log_{10}$ -transformed) of *L. monocytogenes* at 37°C in i) MRS (control) (◆), ii) CFS (▲), iii) CFS adjusted to pH 6.5 (×), iv) CFS supplemented with 0.5mg/mL of catalase (+) and v) CFS supplemented with 1mg/mL of protease (●). CFS of *L. carnosum* is collected from aerobic growth conditions (a) and under vacuum (b). In this Figure, the average viable counts of the duplicates are shown.

Tables

Table 1. Parameter estimates from the Dens et al. model for *L. monocytogenes* and *L. carnosum*, their standard errors (SE) and Mean Squared Error (MSE) of the model fit.

Table 2. Parameter estimates from the Baranyi and Roberts model for *L. monocytogenes* and *L. carnosum*, their standard errors (SE) and Mean Squared Error (MSE) of the model fit.

Table 3. The difference between the onset time of *L. monocytogenes* ($t_{L.monoc}$) and *L. carnosum* ($t_{L.carn}$) expressed as a percentage of the onset time of *L. carnosum* ((%) $t_{L.monoc-decrease}$)

Figure 1.1

Figure 1.2

Figure 2

Figure 3

Figure 4

Table 1

Species	T (°C)	[<i>L. carnosum</i>] (CFU/g)	PARAMETER ESTIMATES ^{1, 2}					MSE
			$\ln(N_0) \pm SE$	$\ln(N_{max}) \pm SE$	$\mu_{max} \pm SE$	$Lag \pm SE$	$a_{12} \pm SE$	
			N_0 $\ln(CFU/g)$	N_{max} $\ln(CFU/g)$	μ_{max} (1/h)	lag (h)	$a_{21} \pm SE$	
<i>L. monocytogenes</i> (1)	4	10 ²	05.14 ± 0.31 ^a	19.14	0.01 ± 0.00 ^a	0.00	0.76 ± 0.13 ^a	0.26
		10 ³	05.41 ± 0.24 ^{ab}	19.14	0.01 ± 0.00 ^a	0.00	0.95 ± 0.15 ^a	0.17
	8	10 ²	05.23 ± 0.33 ^a	19.14	0.03 ± 0.00 ^{ab}	0.00	0.64 ± 0.10 ^a	0.33
		10 ³	05.89 ± 0.42 ^{abc}	19.14	0.01 ± 0.00 ^a	0.00	0.89 ± 0.15 ^a	0.45
	12	10 ²	05.42 ± 0.96 ^{ab}	19.14	0.08 ± 0.02 ^{bc}	0.00	0.53 ± 0.19 ^a	1.74
		10 ³	06.44 ± 0.33 ^c	19.14	0.16 ± 0.08 ^e	42.25 ± 0.03	0.59 ± 0.09 ^a	0.36
		10 ⁴	06.11 ± 0.42 ^{bc}	19.14	0.02 ± 0.01 ^a	0.00	0.45 ± 0.04 ^a	0.26
	25	10 ²	05.46 ± 0.21 ^{ab}	19.14	0.15 ± 0.02 ^{de}	0.00	3.35 ± 0.82 ^b	0.20
		10 ³	05.62 ± 0.20 ^{ab}	19.14	0.09 ± 0.02 ^{cd}	0.00	2.42 ± 0.56 ^b	0.17
<i>L. carnosum</i> (2)	4	10 ²	05.94 ± 0.52 ^a	19.45 ± 0.16 ^c	0.07 ± 0.00 ^a	07.80 ± 14.26	0.00	0.26
		10 ³	07.10 ± 0.26 ^b	19.54 ± 0.14 ^c	0.06 ± 0.00 ^a	0.00	0.00	0.17
	8	10 ²	06.79 ± 0.37 ^{ab}	19.71 ± 0.16 ^{cde}	0.11 ± 0.01 ^a	0.00	0.00	0.33
		10 ³	07.25 ± 0.46 ^b	19.58 ± 0.16 ^{cd}	0.10 ± 0.01 ^a	0.00	0.00	0.45
	12	10 ²	08.47 ± 1.03 ^c	19.88 ± 0.37 ^{de}	0.17 ± 0.03 ^a	0.00	0.00	1.74
		10 ³	10.75 ± 0.47 ^e	19.71 ± 0.16 ^{cde}	0.16 ± 0.02 ^a	0.00	0.00	0.36
		10 ⁴	10.75 ± 0.45 ^e	20.02 ± 0.08 ^e	0.18 ± 0.01 ^a	0.00	0.00	0.26
	25	10 ²	09.88 ± 0.22 ^d	17.94 ± 0.24 ^a	0.38 ± 0.02 ^a	0.00	0.00	0.20
		10 ³	11.87 ± 0.21 ^f	18.25 ± 0.19 ^b	0.33 ± 0.02 ^a	0.00	0.00	0.17

¹For each parameter and species, values bearing different superscripts (no characters in common) are significantly different ($P \leq 0.05$)

²Parameter values without SE were fixed to the values shown in the table (see results)

Table 2

Species	T (°C)	[<i>L. carnosum</i>] (CFU/g)	PARAMETER ESTIMATES ^{1, 2}				MSE
			$\ln(N_0) \pm \text{SE } N_0$ $\ln(\text{CFU/g})$	$\ln(N_{\max}) \pm \text{SE } N_{\max}$ $\ln(\text{CFU/g})$	$\mu_{\max} \pm \text{SE } \mu_{\max}$ (1/h)	Lag $\pm \text{SE lag}$ (h)	
<i>L. monocytogenes</i> (1)	4	0	04.95 $\pm$ 0.51 ^{ab}	17.79 $\pm$ 1.79 ^d	0.02 $\pm$ 0.00 ^a	50.22 $\pm$ 40.91 ^a	0.54
		10 ²	05.02 $\pm$ 0.44 ^{abc}	07.69 $\pm$ 0.13 ^b	0.04 $\pm$ 0.02 ^a	46.97 $\pm$ 33.34 ^a	0.26
		10 ³	06.32 $\pm$ 0.60 ^c	06.32 $\pm$ 0.60 ^a	0.00	0.00	0.36
	8	0	05.08 $\pm$ 0.25 ^{abc}	19.14 $\pm$ 0.22 ^e	0.04 $\pm$ 0.00 ^a	0.00	0.33
		10 ²	05.32 $\pm$ 0.70 ^{abc}	07.70 $\pm$ 0.20 ^b	0.09 $\pm$ 0.09 ^a	31.64 $\pm$ 27.18 ^a	0.65
		10 ³	04.88 $\pm$ 1.00 ^{ab}	06.86 $\pm$ 0.20 ^{ab}	0.09 $\pm$ 0.09 ^a	0.00	1.03
	12	0	05.39 $\pm$ 0.28 ^{abc}	19.11 $\pm$ 0.26 ^e	0.05 $\pm$ 0.00 ^a	0.00	0.62
		10 ²	06.06 $\pm$ 2.09 ^{bc}	09.33 $\pm$ 0.53 ^c	0.32 $\pm$ 0.83 ^a	23.18 $\pm$ 17.97 ^a	4.42
		10 ³	05.85 $\pm$ 0.86 ^{abc}	07.79 $\pm$ 0.24 ^b	0.07 $\pm$ 0.11 ^a	08.90 $\pm$ 48.67 ^a	0.75
		10 ⁴	06.32 $\pm$ 0.34 ^c	06.67 $\pm$ 0.09 ^a	1.17 $\pm$ 35.81 ^a	45.86 $\pm$ 7.14 ^a	0.35
	25	0	04.59 $\pm$ 0.26 ^a	18.82 $\pm$ 0.30 ^e	0.40 $\pm$ 0.02 ^a	0.00	0.38
		10 ²	05.84 $\pm$ 0.23 ^{abc}	08.66 $\pm$ 0.21 ^c	0.29 $\pm$ 0.09 ^a	08.11 $\pm$ 2.68 ^a	0.14
		10 ³	05.99 $\pm$ 0.20 ^{bc}	07.62 $\pm$ 0.20 ^b	0.32 $\pm$ 0.20 ^a	12.74 $\pm$ 3.35 ^a	0.17
<i>L. carnosum</i> (2)	4	10 ²	05.93 $\pm$ 0.41 ^a	19.49 $\pm$ 0.12 ^c	0.07 $\pm$ 0.02 ^a	05.85 $\pm$ 30.66	0.22
		10 ³	07.11 $\pm$ 0.20 ^{bc}	19.55 $\pm$ 0.11 ^{cd}	0.06 $\pm$ 0.00 ^a	0.00	0.11
	8	10 ²	06.84 $\pm$ 0.20 ^b	19.72 $\pm$ 0.09 ^{de}	0.11 $\pm$ 0.00 ^{ab}	0.00	0.10
		10 ³	07.56 $\pm$ 0.32 ^{cd}	19.62 $\pm$ 0.12 ^{cde}	0.10 $\pm$ 0.00 ^a	0.00	0.26
	12	10 ²	07.97 $\pm$ 0.40 ^d	19.80 $\pm$ 0.13 ^e	0.19 $\pm$ 0.01 ^{bc}	0.00	0.24
		10 ³	10.64 $\pm$ 0.44 ^f	19.68 $\pm$ 0.15 ^{cde}	0.17 $\pm$ 0.02 ^e	0.00	0.31
		10 ⁴	10.68 $\pm$ 0.46 ^f	20.03 $\pm$ 0.08 ^f	0.18 $\pm$ 0.01 ^a	0.00	0.27
	25	10 ²	09.83 $\pm$ 0.24 ^e	17.86 $\pm$ 0.25 ^a	0.39 $\pm$ 0.03 ^{de}	0.00	0.23
		10 ³	11.80 $\pm$ 0.21 ^g	18.18 $\pm$ 0.18 ^b	0.34 $\pm$ 0.03 ^{cd}	0.00	0.16

¹For each parameter and species, values bearing different superscripts (no characters in common) are significantly different (P $\leq$ 0.05)

²Parameter values without SE were fixed to the values shown in the table (see results)

Table 3

T (°C)	[<i>L. carnosum</i>] (CFU/g)	Difference between the onset time of <i>L. monocytogenes</i> and <i>L. carnosum</i> expressed as a percentage of the onset time of <i>L. carnosum</i> (%)
4	10 ²	45
	10 ³	96
8	10 ²	48
	10 ³	71
12	10 ²	45
	10 ³	31
	10 ⁴	-80
25	10 ²	12
	10 ³	2