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**Effect of food microstructure on growth dynamics of *Listeria monocytogenes*
in fish-based model systems**

**Davy Verheyen^{a,b,c}, Araceli Bolívar^d, Fernando Pérez-Rodríguez^d, Maria Baka^{a,b,c},
Torstein Skåra^e, Jan F. Van Impe^{a,b,c}**

^aBioTeC+ - Chemical and Biochemical Process Technology and Control, KU Leuven,
Gebroeders de Smetstraat 1, 9000 Gent, Belgium

^bOPTEC, Optimization in Engineering Center-of-Excellence, KU Leuven, Belgium,

^cCPMF², Flemish Cluster Predictive Microbiology in Foods - www.cpmf2.be

^dDepartment of Food Science and Technology, International Campus of Excellence in the
AgriFood Sector, University of Córdoba, C-1, 14014 Córdoba, Spain

^eNofima, P.O. Box 8034, 4068 Stavanger, Norway

davy.verheyen@kuleuven.be, t12bocaa@uco.es, b42perof@uco.es, maria.baka@kuleuven.be,
torstein.skara@nofima.no, jan.vanimpe@kuleuven.be

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Correspondence to:

Prof. J. F. M. Van Impe

Chemical and Biochemical Process Technology and Control (BioTeC+)

Department of Chemical Engineering, KU Leuven

Gebroeders de Smetstraat 1, B-9000 Gent (Belgium)

jan.vanimpe@kuleuven.be

Tel: +32-16-32.14.66

1 **ABSTRACT**

2 Traditionally, predictive growth models for food pathogens are developed based on
3 experiments in broth media, resulting in models which do not incorporate the
4 influence of food microstructure. The use of model systems with various
5 microstructures is a promising concept to get more insight into the influence of food
6 microstructure on microbial dynamics. By means of minimal variation of
7 compositional and physicochemical factors, these model systems can be used to study
8 the isolated effect of certain microstructural aspects on microbial growth, survival and
9 inactivation.

10
11 In this study, the isolated effect on microbial growth dynamics of *Listeria*
12 *monocytogenes* of two food microstructural aspects and one aspect influenced by food
13 microstructure were investigated, i.e., the nature of the food matrix, the presence of
14 fat droplets, and microorganism growth morphology, respectively. To this extent,
15 fish-based model systems with various microstructures were used, i.e., a liquid, a
16 second more viscous liquid system containing xanthan gum, an emulsion, an aqueous
17 gel, and a gelled emulsion. Growth experiments were conducted at 4 and 10°C, both
18 using homogeneous and surface inoculation (only for the gelled systems).

19
20 Results regarding the influence of the growth morphology indicated that the lag phase
21 of planktonic cells in the liquid system was similar to the lag phase of submerged
22 colonies in the xanthan system. The lag phase of submerged colonies in each gelled
23 system was considerably longer than the lag phase of surface colonies on these
24 respective systems. The maximum specific growth rate of planktonic cells in the

liquid system was significantly lower than for submerged colonies in the xanthan system at 10°C, while no significant differences were observed at 4°C. The maximum cell density was higher for submerged colonies than for surface colonies. The nature of the food matrix only exerted an influence on the maximum specific growth rate, which was significantly higher in the viscous systems than in the gelled systems. The presence of a small amount of fat droplets improved the growth of *L. monocytogenes* at 4°C, resulting in a shorter lag phase and a higher maximum specific growth rate. The obtained results could be useful in the determination of a set of suitable microstructural parameters for future predictive models that incorporate the influence of food microstructure on microbial dynamics.

Keywords: Food safety, Predictive microbiology, Structure, Microbial growth.

39 1 INTRODUCTION

40 *Listeria monocytogenes* is a foodborne pathogen of major concern in ready-to-eat
41 (RTE) products (EFSA, 2013). To ensure the microbiological safety of these products,
42 current EU regulation proposes predictive microbiology as a valuable tool to be used
43 by food business operators for conducting shelf-life studies on *L. monocytogenes* in
44 RTE foods (EC, 2005; EURL Lm, 2008). In Europe, the prevalence of *L.*
45 *monocytogenes* remains high in RTE fish and fishery products at processing and retail
46 (EFSA, 2016). For this reason, considerable efforts have been made during the last
47 decade to develop models that are able to provide accurate predictions for this
48 pathogen in minimally processed fish products (Couvert et al., 2010; Mejlholm and
49 Dalgaard, 2007, 2009; Mejlholm et al., 2015).

50

51 Since most predictive models describing the impact of several factors on microbial
52 kinetics are based on growth in broth media, the effect of food microstructure on
53 microbial behaviour, generally considered as an additional stress factor which lowers
54 growth rates, is often neglected (Baka et al., 2017a; Mertens et al., 2011; Pérez-
55 Rodríguez and Valero, 2013). Therefore, challenge tests are usually required to assess
56 the growth of a target microorganism directly in/on the target food product (Mejlholm
57 et al., 2010). Challenge tests, however, are expensive and time-consuming, and the
58 inherent variability of batches, together with the presence of accompanying
59 microbiota often hamper a precise quantification of the microorganism as well as a
60 suitable study of the combined effects of food intrinsic factors (Augustin et al., 2011).
61 The use of food-based model systems can help to effectively describe microbial
62 behaviour under conditions that mimic the real food environment, although

63 validations are always recommended before their use in other specific food systems
64 (EURL Lm, 2014; te Giffel and Zwietering, 1999).
65
66 Heertje (1993) defined food microstructure as “the spatial arrangement of the
67 structural elements and their interactions”. All structural elements (e.g., droplets, air
68 cells, granules, crystals, strands, micelles, and interfaces), as well as the resulting
69 architecture, should be taken into account when the microstructure of a food product
70 is investigated (Aguilera, 2009; Heertje, 1993). In literature, the influence on
71 microbial dynamics of several food microstructural aspects is reported (Smet et al.,
72 2015a). Among those aspects, the nature of the food matrix (e.g., liquid/viscous,
73 gelled/solid) not only affects growth dynamics but also influences the growth
74 morphology of the microorganisms (Skandamis and Jeanson, 2015; Wilson et al.,
75 2002). In food products, microorganisms mostly occur in three different growth
76 morphologies or a combination of those, i.e., planktonic cells, submerged colonies
77 and surface colonies (Wimpenny, 1992). In liquid or viscous systems, the growth
78 morphology is typically characterised as freely-dispersed planktonic cells, enabling
79 taxis to preferred nutrient-rich locations of the food (Smet et al., 2015a; Wilson et al.,
80 2002). In foods which are gelled due to the presence of certain gelling agents (e.g.,
81 gelatin, starch, gums), bacteria are immobilised and constrained to grow as three-
82 dimensional submerged colonies inside the food matrix, reducing the diffusion of
83 nutrients and metabolites to and from the colonies (Antwi et al., 2006; Brocklehurst et
84 al., 1997; Skandamis and Jeanson, 2015). Bacteria on food surfaces grow as three-
85 dimensional colonies in which replenishment of nutrients only takes place from the
86 bottom or perimeter of the colony, resulting in starvation and self-intoxication of cells
87 in the centre of the colony (Skandamis and Jeanson, 2015; Wilson et al., 2002). It is

generally assumed that the bacterial growth rate in these different growth morphologies follows the order: planktonic cells $\geq$ submerged colonies $\geq$ surface colonies (Brocklehurst et al., 1997). However, recent studies have demonstrated a more complex relationship as a function of a plethora of different factors, e.g., temperature, food composition, food microstructure and related rheological properties, the presence of fat droplets, the target microorganism, oxygen availability, and physicochemical properties such as pH and a_w (Aspridou et al., 2014; Baka et al., 2017a, b; Lobete et al., 2017; Smet et al., 2015a, b). Food products can be classified in liquids, aqueous gels, emulsions, gelled emulsions, and surfaces (Wilson et al., 2002). In order to assess the influence of each level of microstructural complexity on microbial growth, recent studies have developed food-based systems covering the whole range in accordance with this classification (Baka et al., 2016, 2017a, 2017b). Baka et al. (2017a) evaluated the effect of different food microstructures on growth kinetics of *L. monocytogenes*. For this purpose, they developed model systems mimicking fish patés which were incubated at refrigerated and abuse storage temperatures. It was observed that surfaces of solid systems may favour *L. monocytogenes* growth under aerobic conditions, while liquid systems without fat at low temperatures exhibited the slowest growth. Similar conclusions were drawn in experiments with Frankfurter sausages (Baka et al., 2016), where it was found that the firmer the solid system, the faster the *L. monocytogenes* growth. These findings indicate that the microstructural effect is complex and seems to be dependent on temperature as well as compositional (i.e. fat presence) and physicochemical factors of the specific food system. However, in the aforementioned studies, the different microstructures were achieved by adding or removing components (i.e., fat, emulsifiers and/or gelling agents) in the model systems, thus resulting in noticeable

differences in compositional and physicochemical aspects. The minimisation of these variations among the model systems would help to clarify and isolate the different microstructural effects on microbial dynamics. In this regard, Verheyen et al. (2017) developed fish-based model systems with minimal variations in physicochemical and compositional aspects, which are suitable for the investigation of the isolated food microstructural effect on *L. monocytogenes* growth dynamics. In search of suitable parameters to include in future predictive models, rheological and structural properties, as well as fat droplet size of the model systems were characterised.

In this study, the isolated effect of food microstructure on growth dynamics of *L. monocytogenes* was investigated, using fish-based model systems (Verheyen et al., 2017), i.e., two liquid systems, an emulsion, an aqueous gel, and a gelled emulsion. More specifically, the effect of *L. monocytogenes* growth morphology, the nature of the food matrix (i.e., viscous vs. gelled food matrix) and the presence of fat droplets was studied on the growth parameters of the Baranyi and Roberts (1994) model. Homogeneous and surface growth experiments were conducted at temperatures of 4 and 10°C, relevant for fish product storage (i.e., refrigeration and abuse temperature, respectively). Based on these experiments, relevant microstructure-related parameters (e.g., rheological properties, properties related to the fat droplets) were suggested for inclusion in future predictive growth models that take into account the influence of food microstructure on microbial dynamics.

2 MATERIALS AND METHODS

2.1 Model system preparation

Fish-based model systems with various microstructures were prepared according to the protocol of Verheyen et al. (2018). Briefly, a set of five different model systems was used, consisting of (i) a liquid system, (ii) xanthan, i.e., a second liquid system containing a small concentration of xanthan gum, (iii) an emulsion, (iv) an aqueous gel, and (v) a gelled emulsion. The xanthan system had an increased viscosity compared to the liquid system, causing the cells to grow as submerged colonies instead of planktonic cells, enabling a further investigation of the influence of growth morphology on microbial dynamics. The microstructural effect was isolated among the different model systems by means of minimal variation in compositional (e.g., the emulsion and gelled emulsion had a fat content of 1%) and physicochemical factors (i.e., pH and a_w). The average pH and a_w of the model systems were 6.36 ± 0.03 and 0.988 ± 0.002 , respectively, being representative for fish-based food products for which the pH mostly ranges from 5.5 to 6.8 (Jay et al., 2005) and the $a_w > 0.938$ (Schmidt and Fontana, 2007). Concerning the viscous systems, the liquid exhibited Newtonian behaviour, while the xanthan and the emulsion exhibited non-Newtonian pseudo-plastic behaviour. The aqueous gel and gelled emulsion were strong gels with a hardness of 1.35 ± 0.07 N and 1.25 ± 0.05 N, respectively. Fat droplet size of the emulsion and gelled emulsion model systems was evenly distributed around 1 μ m (Verheyen et al., 2018). Homogeneous growth experiments were performed in small vials (4mL, 45 x 14.7 mm, BGB Analytik Benelux B.V., Harderwijk, the Netherlands) containing 1 mL of viscous or structured medium, while surface growth experiments were performed in Petri dishes (55 x 12 mm, Anicrin S.R.L., Scorzè, Italy) containing 3 mL of structured medium, with a surface area of approximately 2.4 cm².

2.2 Microorganisms and preculture conditions

L. monocytogenes strains LMG 23773, LMG 23774 (both isolated from smoked salmon), and LMG 26484 (isolated from tuna salad) were acquired from the BCCM/LMG bacteria collection of Ghent University in Belgium. Stock cultures were stored at -80°C in Brain Heart Infusion Broth (BHI, VWR International, Leuven, Belgium) and 20% (v/v) glycerol (Acros Organics, NJ, USA). For the inoculum preparation, a fresh purity plate was prepared for each strain from the frozen stock culture by spreading a loopful onto a BHI Agar plate (1.4% (w/v), Agar Technical No3, Oxoid Ltd., Basingstoke, UK), incubated at 30°C for 24 h. Subsequently, one colony from each purity plate was transferred into 20 mL of BHI in separate 50 mL Erlenmeyers. After incubating for 24 h at 30°C under static conditions, 20 µL of each stationary-phase culture was inoculated into 20 mL of fresh BHI and incubated for 24 h under the same conditions, resulting in early stationary-phase cultures with a cell density of approximately 10⁹ CFU/mL for each of the three strains. From each culture, 10 mL was collected in a 100 mL Erlenmeyer under aseptic conditions and mixed, resulting in a *L. monocytogenes* strain cocktail with a cell density of approximately 10⁹ CFU/mL. Subjecting every strain cocktail to the same preculture conditions prior to model system inoculation ensured that all growth experiments were started from microorganisms with the same initial physiological state.

2.3 Inoculation and culture conditions

Model systems were inoculated with the *L. monocytogenes* strain cocktail, as described briefly in Verheyen et al. (2018). For homogeneous growth experiments, systems were inoculated to a cell density of 10² CFU/mL, using a NaCl solution of

0.90% (w/v) as dilution medium. For surface growth experiments, model systems prepared 24 h beforehand and stored at the temperature of the growth experiment to allow the gel to solidify, were inoculated to a cell density of 10^2 CFU/cm², using an L-shaped spreader. These inoculated model systems were incubated at temperatures of 4 or 10°C. Samples were taken in duplicate at different time intervals (i.e., approximately every 24 h). For the viscous systems (i.e., liquid, xanthan and emulsion), serial decimal dilutions were directly prepared from an aliquot of the samples, using the NaCl solution and afterwards plated on BHI agar plates, employing the drop technique to carefully deposit three drops of 20 µL on the plate for every dilution (Herigstad et al., 2001). For the gelled systems (i.e., aqueous gel and gelled emulsion), the protocol of Hamoud-Agha et al. (2013) was used. More specifically, gels were transferred from the vials/Petri dishes into sterile stomacher bags under aseptic conditions. Subsequently, the vials/Petri dishes were rinsed and drained into the bag with 1.5 and 4.5 mL of Phosphate Buffered Saline (PBS, Sigma Aldrich, MO, USA), respectively. Afterwards, 1.5 and 4.5 mL of McIlkaine buffer (0.2 M Na₂HPO₄, Sigma Aldrich, MO, USA; 0.1 M citric acid, Acros Organics, NJ, USA) was added to each bag, for samples originating from the vials and Petri dishes, respectively. The mixture was homogenised for 10 min (basic masticator, Led techno, Heusden-Zolder, Belgium), after which serial decimal dilutions were prepared and plated similarly to the viscous systems. The plates were incubated at 30°C for approximately 30 h prior to enumeration. Each experiment was performed independently, in triplicate.

2.4 Modelling and parameter estimation

The model of Baranyi and Roberts (1994), represented by Equations 1, 2 and 3, was fitted to the experimental data.

210

$$\frac{dN(t)}{dt} = \left(\frac{Q}{1+Q} \right) \mu_{max} \left(1 - \frac{N(t)}{N_{max}} \right) N(t) \quad (1)$$

$$\frac{dQ(t)}{dt} = \mu_{max} \cdot Q(t) \quad (2)$$

$$\lambda \cdot \mu_{max} = \ln \left(1 + \frac{1}{Q(0)} \right) \quad (3)$$

211

212 With $N(t)$ [CFU/mL or cm²], the cell density at time t ; N_0 [CFU/mL or cm²], the initial
 213 cell density at time 0; N_{max} [CFU/mL or cm²], the maximum cell density; μ_{max} [1/h],
 214 the maximum specific growth rate; Q [-], the physiological state of the cells; $Q(0)$, a
 215 measure of the initial physiological state of the cells; and λ [h], the lag time of the
 216 cells. Since the experiments in this study were conducted at static temperature
 217 conditions, the model was simplified to:

218

$$\ln(N(t)) = \ln(N(0)) + \mu_{max} \cdot A(t) - \ln \left(1 + \frac{e^{\mu_{max} \cdot A(t)} - 1}{e^{(N_{max} - N(0))}} \right) \quad (4)$$

$$A(t) = t + \frac{1}{\mu_{max}} \ln \left(\frac{e^{-\mu_{max} \cdot t} + Q(0)}{1 + Q(0)} \right). \quad (5)$$

219

220

221 Model parameters were estimated from the set of experimental data via the
 222 minimisation of the sum of squared errors, using the lsqnonlin routine of the
 223 Optimisation Toolbox of Matlab version R2016b (The Mathworks Inc.). Standard
 224 errors of parameter estimates were calculated from the Jacobian matrix. A global
 225 estimation procedure was standardised for each model system.

226

227 **2.5 Statistical analysis**

228 Significant differences were determined using analysis of variance (ANOVA, single
229 variance) test at a 95.0% confidence level ($\alpha = 0.05$). Fisher's Least Significant
230 Difference (LSD) test was used to distinguish which means were significantly
231 different from others. The analyses were performed using the anova1 routine of the
232 Statistical Toolbox of Matlab version R2016b (The Mathworks, Inc.). Test statistics
233 were regarded as significant when $P \leq 0.05$.

234

3 RESULTS AND DISCUSSION

Figure 1 represents an overview of the different microstructural aspects that were investigated: (i) effect of growth morphology, (ii) effect of the nature of the food matrix, and (iii) the effect of fat droplets. In this regard, it was considered that the liquid model system supported planktonic growth, while the xanthan, emulsion, aqueous gel and gelled emulsion model systems supported colony growth (Verheyen et al., 2018). Growth dynamics of *L. monocytogenes* in/on the different model systems are illustrated in Figure 2 and Figure 3 for growth at 4 and 10°C, respectively. Figure 4, Figure 5, and Figure 6 demonstrate corresponding parameter estimates of the Baranyi and Roberts (1994) model for the lag time (λ), the maximum specific growth rate (μ_{max}), and the maximum cell density (N_{max}), respectively. In order to investigate the isolated effect of microstructure on *L. monocytogenes* growth dynamics, growth parameters are compared for the different model systems.

3.1 Effect of growth morphology

The effect of growth morphology was investigated by comparing growth dynamics of *L. monocytogenes* between systems where the growth morphology is the sole significant difference (i.e., planktonic cells in liquid vs. submerged colonies in xanthan and submerged colonies in vs. surface colonies on the gelled systems).

Figure 4 illustrates that the lag phase λ in the liquid system was significantly longer than in the xanthan system at 4°C, while no significant differences were detected at 10°C. However, as observed in Figure 2 and confirmed by the raw data (not shown), the viable cell density in the liquid and xanthan system at 4°C remained constant for a similar period of time (i.e., approximately 100 h), suggesting that the statistical

differences between the respective lag phases, as illustrated in Figure 4, were most likely caused by the fitting of the growth model to the data. Therefore, the lag phase λ was probably not influenced by the growth morphology of the cells at both temperatures. These findings are in contradiction with the results of Meldrum et al. (2003) for growth of *L. monocytogenes* at 10°C; stating that planktonic cells exhibit a shorter lag phase than submerged colonies. The different behaviour in this study could be due to the fact that experiments were conducted at static conditions (i.e., the tubes were not shaken during the growth experiments). As planktonic cells sediment over time, they are exposed to lower oxygen and nutrient concentrations as compared to the submerged colonies which stay in the bulk of the system (Smet et al., 2015a, b). Furthermore, Figure 4 also shows that the lag phase λ of submerged colonies in each gelled system was considerably longer than λ of surface colonies on these respective systems (although only exhibiting statistical differences for the aqueous gel), both at 4 and 10°C. *L. monocytogenes* is a facultative anaerobe (Farber and Peterkin, 1991), but its growth is enhanced by aerobic conditions (Pine et al., 1989; Romick et al., 1996) and even more so when there is a better oxygen availability (Smet et al., 2015a). Therefore, oxygen availability is the most probable explanation for this phenomenon, since submerged colonies have less available oxygen than surface colonies (Baka et al., 2017a).

Figure 5 shows that the maximum specific growth rate μ_{max} in the liquid system was significantly lower than in the xanthan system at 10°C, while no significant differences were seen at 4°C. The static conditions (i.e., not shaking of the tubes) of the experiment are the most probable explanation for this phenomenon as well, since the greater oxygen availability allows a similar or higher μ_{max} of *L. monocytogenes*

(Smet et al., 2015a). When comparing the maximum specific growth rate μ_{max} of submerged and surface colonies in gelled systems, it becomes clear that μ_{max} of surface colonies was significantly higher, both at 4 and 10°C. Similar to what was concluded for the lag phase, the lower amount of oxygen available for submerged colonies is the most likely cause of this phenomenon (Baka et al., 2017a).

In Figure 6, it can be observed that the maximum cell density N_{max} of planktonic colonies was not significantly different from N_{max} of submerged colonies. However, the maximum cell density N_{max} was considerably lower for surface colonies than for submerged colonies, both at 4 and 10°C, suggesting that, even though oxygen availability is greater for surface colonies than submerged colonies, the maximum cell density N_{max} of surface colonies is limited by the exclusive replenishment of nutrients from the bottom of the colony (Skandamis and Jeanson, 2015).

3.2 Effect of the nature of the food matrix

In this section, the effect of a gelled matrix as compared to a viscous matrix (i.e., aqueous gel vs. xanthan and gelled emulsion vs. emulsion) on the growth dynamics of submerged colonies is investigated.

Figure 4 shows that the lag phase λ of submerged colonies was slightly longer in a gelled matrix than in a viscous matrix, although no significant differences could be observed, both at 4 and 10°C. Consequently, the effect of gelation on the duration of the lag phase, although present, is less evident than the effect of growth morphology.

Figure 5 illustrates that gelation had a significant effect on μ_{max} , as the maximum specific growth rate μ_{max} was higher in viscous systems than in gelled systems, both at 4 and 10°C. This was probably caused by the enhanced nutrient, oxygen and metabolite diffusion in the viscous systems as compared to the gelled systems, which has been reported extensively in literature (Antwi et al., 2006; Brocklehurst et al., 1997; Wilson et al., 2002, Wimpenny et al., 1981, 1995). In the current study, however, the difference is observed between submerged colonies in viscous and gelled systems, adding a new dimension to this knowledge. Previous studies which investigated the effect of a gelled matrix on microbial growth (e.g., Antwi et al., 2006, 2007; Brocklehurst et al., 1997; Theys et al., 2008) only compared planktonic cells in a liquid system and submerged colonies in a gelled system, which also introduces the effect of differences in growth morphology.

Figure 6 indicates that the gelation had no significant influence on N_{max} of submerged colonies, which is due to the absence of significant compositional differences among the model systems (Verheyen et al., 2018).

3.3 Effect of presence of fat droplets

The effect of the presence of fat droplets on *L. monocytogenes* growth dynamics was investigated by comparing growth dynamics in systems among which the only difference is the presence of fat droplets (i.e., xanthan vs. emulsion, aqueous gel vs. gelled emulsion, aqueous gel (surface) vs. gelled emulsion (surface)).

The results presented in Figure 4 indicate that the presence of fat droplets at the level used in these experiments did not show any significant influence on λ at 10°C. At

4°C, on the other hand, the lag phase λ was always significantly shorter when fat droplets were present, although only for submerged colonies. For surface colonies, the presence of fat droplets had no significant influence on λ . Therefore, it can be concluded that, at low temperatures, submerged colonies of *L. monocytogenes* growing in a matrix containing fat droplets, have a shorter lag phase than submerged colonies in a matrix without fat droplets. Similar findings (although for planktonic *L. monocytogenes* cells) were reported by Baka et al. (2017a), who suggested that fat presence in emulsions at 4°C acts as a cryoprotective agent for the cells.

When comparing the influence of fat droplets on μ_{max} in Figure 5, it can be observed that fat droplets also led to an increase of μ_{max} (only) for submerged colonies of *L. monocytogenes* at 4°C. The combination of this finding and the lag phase reduction already discussed, suggests that the presence of a low amount of fat droplets in foods is beneficial for colony growth of *L. monocytogenes* at low temperatures. This conclusion is rather counter-intuitive, since the presence of fat in food products is mostly known as a growth-restricting factor (Wilson et al., 2002). In this study, however, the fat concentration was very low and had no significant influence on the a_w of the model systems (Verheyen et al., 2018), which may contribute to explaining why there was no growth-limiting effect. Nevertheless, the beneficial effect of the presence of fat droplets on the growth (i.e., μ_{max} and λ) of *L. monocytogenes* at low temperatures has, to the best knowledge of the authors, not been described in literature. Taking into account the important consequences for food safety, a more detailed investigation of this effect in future studies is essential.

Similar to what was observed for effect of the gelled matrix, Figure 6 indicates that the presence of fat droplets has no influence on N_{max} of *L. monocytogenes*. As a consequence, the growth morphology is shown to be the only microstructural aspect that influences N_{max} .

3.4 Future modelling perspectives

The findings from the previous sections can be used to suggest suitable parameters for the inclusion of the food microstructural influence into predictive growth models for pathogens. In this regard, future studies could focus on developing secondary models which describe the effect of those parameters on the microbial growth parameters (e.g., λ , μ_{max} , N_{max}). In order to incorporate the food microstructural influence into primary growth models such as the Baranyi and Roberts (1994) model, the parameters from those models can be replaced by the corresponding secondary models (Theys et al., 2008; Valdramidis et al., 2005).

It was illustrated that the growth morphology of microorganisms can exert, in some cases, an important influence on growth dynamics (i.e., λ , μ_{max} , N_{max}). Therefore, future predictive models that incorporate the influence of food microstructure need to include a growth morphology factor which would incorporate all parameters related to growth morphology that influence growth dynamics. This factor should include a temperature dependency, as a complex relation between the effect of growth morphology and temperature was found in this study. Since the sedimentation rate also seems to be an important influencing factor for planktonic cell growth, the growth morphology factor could also incorporate the viscosity of the food product, as viscosity is known to affect the sedimentation rate (Thies-Weesie et al., 1996).

A significant influence of the nature of the food matrix (i.e., viscous or gelled) on microbial growth dynamics was also established, especially on μ_{max} . In this regard, Antwi et al. (2006, 2007) and Theys et al. (2008) already included the gelling agent concentration (i.e., gelatin concentration) of their model systems as a parameter into their growth models in order to take the influence of food matrix structure into account. However, since each specific gelling agent influences food microstructure and the corresponding effect on microbial dynamics in a different way, isolating a set of universal parameters that identifies the effect of food microstructure on microbial growth dynamics would be more suitable (Aspridou et al., 2014). In this regard, variation in nutrient, oxygen and metabolite diffusion can explain the differences in behaviour among viscous and gelled systems (Robins & Wilson, 1994). These diffusion differences are affected by the rheological properties of the food matrix (Gould & Christian, 1988; Stecchini et al., 1998). As to which rheological properties could be included into predictive models, the influence on microbial growth of the properties determined for the current model systems in Verheyen et al. (2018) could be investigated, e.g., viscosity parameters of the Power law model (Reiner, 1926) for viscous systems, and the storage (G') and loss (G'') modulus for gelled systems. To this end, the composition of the current set of model systems would need to be adapted (while still minimising compositional and physicochemical differences) in order to create a set of model systems with varying rheological properties. However, even though the inclusion of matrix-related factors in predictive models based on artificial or simplified systems could enhance their accuracy, validation in actual food products is necessary.

Concerning the findings of Section 3.3, predictive models could also include a factor which expresses the influence of the presence of fat droplets on λ and μ_{max} . However, further research into the exact influencing mechanics would first be necessary. The fat droplet size and the fat concentration are examples of possible aspects to be investigated. In this regard, the fat concentration of food products is known to have an influence on microbial growth (Akalın, et al., 2007; Shakerian et al., 2014) and inactivation (Juneja et al., 2001) dynamics, indicating the possibility of including the fat concentration in predictive models. Although research has been conducted on the antimicrobial effect of oil-in-water emulsions with different fat droplet sizes which were loaded with phenolic antimicrobials (Terjung et al., 2012), the influence of fat droplet size of oil-in-water emulsions on microbial growth dynamics has not been studied so far to the best knowledge of the authors. Consequently, further research on growth dynamics of *L. monocytogenes* in emulsions and gelled emulsions with different mean droplet sizes should first be conducted to check for an influence on microbial dynamics. Since a complex relation between the influence of the presence of fat droplets and growth temperature was demonstrated in this study, the factor which expresses this fat droplet influence could also be dependent on the growth temperature. Due to the temperature dependence of the influence of several microstructural aspects on microbial growth dynamics, sufficient care should be taken when determining the model structure (and associated model parameters) of the different factors to be included in future predictive models.

4 CONCLUSIONS

In this study, it was illustrated that the three assessed microstructural aspects have a significant influence on microbial growth dynamics of *L. monocytogenes*, affecting, at least, some of the kinetic parameters λ , μ_{max} and N_{max} . The variations in oxygen availability and nutrient diffusions could be the main causes of the observed differences for the growth morphology and the nature of the food matrix. In the case of emulsion systems, it can be concluded that the formulation of fat at low concentration can enhance growth. However, further research will be needed to elucidate the effect of other fat concentrations as well as in the fat droplet size distribution on growth.

This study was a first step in the determination of suitable microstructural parameters for future predictive models that incorporate the influence of food microstructure on microbial dynamics. In order to develop those improved predictive models, more research should be conducted to determine into more detail the influence of the suggested parameters, as well as to get a better understanding of the influencing mechanics.

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FIGURE CAPTIONS

Figure 1: Schematic overview of the different model systems and their respective microstructural characteristics. The investigation of the microstructural aspects is divided into different aspects, i.e., (i) effect of growth morphology, (ii) effect of the nature of the food matrix, and (iii) the effect of fat droplets.

Figure 2: Growth curves of *L. monocytogenes* at 4°C in the different model systems. Symbols correspond to the experimental data (x, ○, +, □, *, ◇, and Δ for the liquid, xanthan, emulsion, aqueous gel, gelled emulsion, aqueous gel (surface), and gelled emulsion (surface), respectively) and lines correspond to the global fit of the Baranyi and Roberts (1994) growth model [$\ln(\text{CFU/mL or cm}^2)$].

Figure 3: Growth curves of *L. monocytogenes* at 10°C in the different model systems. Symbols correspond to the experimental data (x, ○, +, □, *, ◇, and Δ for the liquid, xanthan, emulsion, aqueous gel, gelled emulsion, aqueous gel (surface), and gelled emulsion (surface), respectively) and lines correspond to the global fit of the Baranyi and Roberts (1994) growth model [$\ln(\text{CFU/mL or cm}^2)$].

Figure 4: Statistical analysis of the lag phase λ , according to the growth model of Baranyi and Roberts (1994) for the different growth experiments with *L. monocytogenes*, both at 4 and 10°C. Model systems are characterised as follows: Liq = liquid, Xan = xanthan, Em = emulsion, AG = aqueous gel, GE = gelled emulsion, AGs = aqueous gel (surface), and GEs = gelled emulsion (surface). Error bars represent the standard error on the estimated parameter values. For the different model systems at the same temperature, bars bearing different uppercase letters are significantly different ($P \leq 0.05$).

Figure 5: Statistical analysis of the maximum specific growth rate μ_{max} , according to the growth model of Baranyi and Roberts (1994) for the different growth experiments with *L. monocytogenes*, both at 4 and 10°C. Model systems are characterised as follows: Liq = liquid, Xan = xanthan, Em = emulsion, AG = aqueous gel, GE = gelled emulsion, AGs = aqueous gel (surface), and GEs = gelled emulsion (surface). Error bars represent the standard error on the estimated parameter values. For the different model systems at the same temperature, bars bearing different uppercase letters are significantly different ($P \leq 0.05$).

Figure 6: Statistical analysis of the maximum cell density N_{max} , according to the growth model of Baranyi and Roberts (1994) for the different growth experiments with *L. monocytogenes*, both at 4 and 10°C. Model systems are characterised as follows: Liq = liquid, Xan = xanthan, Em = emulsion, AG = aqueous gel, GE = gelled emulsion, AGs = aqueous gel (surface), and GEs = gelled emulsion (surface). Error bars represent the standard error on the estimated parameter values. For the different model systems at the same temperature, bars bearing different uppercase letters are significantly different ($P \leq 0.05$).

672 **HIGHLIGHTS**

- 673 •*Listeria* growth is similar for planktonic cells and submerged colonies
- 674 •Surface colonies exhibit higher growth rate than submerged colonies
- 675 • N_{max} of surface colonies is lower than for planktonic cells and submerged colonies
- 676 •Growth rate in viscous systems is higher than in gelled systems
- 677 •Small amount of fat droplets enhances *Listeria* growth at low temperature
- 678