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Influence of food intrinsic complexity on *Listeria monocytogenes* growth in/on vacuum-packed model systems at suboptimal temperatures

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ABSTRACT

Food intrinsic factors e.g., food (micro)structure, compositional and physicochemical aspects, which are mutually dependent, influence microbial growth. While the effect of composition and physicochemical properties on microbial growth has been thoroughly assessed and characterised, the role of food (micro)structure still remains unravelled. Most studies on food (micro)structure focus on comparing planktonic growth in liquid (microbiological) media with colonial growth in/on solid-like systems or on real food surfaces. However, foods are not only liquids or solids; they can also be emulsions or gelled emulsions and have complex compositions. In this study, *Listeria monocytogenes* growth was studied on the whole spectrum of (micro)structure, in terms of food (model) systems. The model systems varied not only in (micro)structure, which was the target of the study, but also in compositional and physicochemical characteristics, which was an inevitable consequence of the (micro)structural variability. The compositional and physicochemical differences were mainly due to the presence or absence of fat and gelling agents. The targeted (micro)structures were: i) liquids, ii) aqueous gels, iii) emulsions and iv) gelled emulsions. Furthermore, the microbial dynamics were studied and compared in/on all these model systems, as well as on a compositionally predefined canned meat, developed in order to have equal compositional level to the gelled emulsion model system and represent a real food system. Frankfurter sausages were the targeted real foods, selected as a case study, to which the canned meat had similar compositional characteristics. All systems were vacuum packed and incubated at 4, 8 and 12°C. The most appropriate protocol for the preparation of the model systems was developed. The pH, water activity and resistance to penetration of the model systems were characterised. Results indicated that low temperature contributes to growth variations among the model systems. Additionally, the firmer the solid system, the faster *L. monocytogenes* grew on it. Finally, it was found that *L. monocytogenes* grows faster on canned meat and real Frankfurters, as found in a previous

study, followed by liquids, aqueous gels, emulsions and gelled emulsions. This observation indicates that all model systems, developed in this study, underestimated *L. monocytogenes* growth. Despite some limitations, model systems are overall advantageous and therefore, their validation is always recommended prior to further use.

Keywords: structure, food intrinsic factors, model systems, meat products, emulsions, gelled systems.

Introduction

Predictive microbiology describes microbial dynamics, i.e., growth or inactivation as a function of environmental factors, by means of mathematical models. Most predictive models have been developed on the basis of experimental data obtained: i) in liquid microbiological media (planktonic growth) or ii) in and/or on real food products (homogeneous or surface inoculation, respectively). On the one hand, food products are not always liquid and often exhibit a more complex structure, which is not considered in the models based on liquid media. Therefore, these models describe well microbial growth in liquid food products, but not necessarily in/on real, or more complex, food systems. Additionally, other food intrinsic factors, e.g., compositional and some physicochemical characteristics, e.g., resistance to penetration are not taken into account when working with liquid microbiological media. On the other hand, challenge tests, where the growth of target microorganisms is directly monitored in/on real food products, and therefore, the effect of food (micro)structure, compositional and physicochemical characteristics is intrinsically present, are expensive and time-consuming (Wareing and Komitopoulou, 2013). Moreover, food products exhibit high batch to batch variability and a pre-treatment is required for the elimination of their background micro-flora, which affects their food (micro)structure and composition, such as dipping in boiling water (Baka et al., 2014), applicable to challenge testing. For the preparation of model systems of various (micro)structures is crucial to ensure reproducibility and exclude the background microflora effect, when this is necessary. Additionally, it is of high importance to develop such systems at which the isolated effect of (micro)structure, composition and physicochemical variability can be studied.

Food (micro)structure has been defined as the layout of particles and spaces between the particles, whilst structure as the shape and size of the material (Mebatsion et al., 2008). Food structure has been classified in five categories (liquids, aqueous gels, emulsions, gelled

emulsions and food surfaces), where representative examples of food products have been proposed for each one of the categories (Wilson et al., 2002) and examples are provided in Figure 1. Therefore, liquids and gelled emulsions are two experimental systems which represent the edges of a wide spectrum of food matrix complexity. The food (micro)structure has been acknowledged to influence microbial growth and the mechanisms of influence have been identified (Brocklehurst et al., 1997; Robins and Wilson, 1994; Wilson et al., 2002). Cells grow planktonically in liquid and oil-in-water emulsion systems, with fat level lower than 83% (Brocklehurst and Wilson, 2000). Nevertheless, in oil-in-water emulsions, the available physical space for growth is limited, in comparison to the liquid systems. In solid-like systems, the mechanisms of nutrients and oxygen transportation are different than in liquids. It has been reported that in solid-like systems, constraints in oxygen availability, and limited water, nutrients, preservatives and metabolites distribution take place (Koutsoumanis et al., 2004; Noriega et al., 2008a, 2008b, 2009; Wilson et al., 2002; Wimpenny and Coombs, 1983). Additionally, solid-like foods confine cells and force them to form colonies. As a consequence, stress is induced to the immobilised cells. Stress is reflected with changes in microbial metabolism, where alterations in growth rate, cell morphology, membrane permeability, superficial tension and osmotic pressure have been observed (Dervakos and Webb, 1991; Meldrum et al., 2003; Wilson et al., 2002). Immobilised cells exhibit substrate and product concentration gradients around the colony, as molecular diffusion is much slower than convection occurring in liquid systems (Brocklehurst et al., 1997; Malakar et al., 2000, 2003; Walker et al., 1997; Wimpenny et al., 1995). Nevertheless, contradictions exist in literature. Solid-like systems have also been reported to enhance survival or even microbial growth (Antwi et al., 2007; Mertens et al., 2011; Wilson et al., 2002).

Numerous studies have been carried out, studying the effect of environmental factors or the concentration of gelling agents, fat and emulsifiers on model systems of various

(micro)structures. These studies include: i) liquids that have been solidified by the addition of gelling agents (Antwi et al., 2006; Brocklehurst et al., 1997; Mertens et al., 2011; Theys et al., 2008), or emulsified by the addition of fat / oil and emulsifiers (Brocklehurst et al., 1993, 1995; Castro et al., 2009; Parker et al., 1995), and ii) food model systems similar to real food systems, such as liver pâté (Farber et al., 1995), minced chicken breast (Noriega et al., 2010a) and cheese (Jeanson et al., 2011). Furthermore, there are studies where microbial growth dynamics have been studied under the same environmental conditions, in liquid microbiological media compared to solidified microbiological media (Boons et al., 2013a, 2013b, 2014; Koutsoumanis et al., 2004; Lebert et al., 2004; Mertens et al., 2011). Significant differences on the growth dynamics of the two types of model systems were found. Consequently, it is of utmost importance to develop model systems, which cover the whole spectrum of structural complexity. Additionally, the microbial dynamics among all the model systems and the target food product need to be studied and compared. Thus, the influence of each level of structural complexity on microbial growth dynamics will be quantified for a specific microorganism and food product. In this study, the effect of food (micro)structure will be assessed, by means of model systems exhibiting variable (micro)structures. The output of the study will be discussed also on the basis of the compositional and physicochemical variations, which inevitably occurred due to the presence or absence of fat and gelling agents at the different model systems. *Listeria monocytogenes* was selected as a model microorganism. This foodborne pathogen has a high fatality rate (15.6%) (EFSA, 2015), and is able to survive under harsh conditions. Therefore, the food industry pays special attention to control this organism at the production line and the final products. This pathogenic species is often related to cases of listeriosis in fish, ready-to-eat (RTE) and dairy products (EFSA, 2014). Frankfurter sausages are RTE meat products, which were among the categories with the highest levels of non-compliance to the regulations (2.5 %) at the processing level in 2012, with respect to the presence of *L. monocytogenes*.

(EFSA, 2014). Frankfurter sausages represent a complex (micro)structure of gelled emulsion systems and were selected as the target food products. The growth dynamics of *L. monocytogenes* will be studied in/on four model systems (liquids, aqueous gels, emulsions, gelled emulsions) and on a food product (canned meat), similar in composition to Frankfurter sausages, at three storage temperatures (4, 8 and 12°C) and under vacuum. The solid meat-based (model) systems will be surface inoculated, vacuum packed and their cell concentration will be monitored at a population level. Surface inoculation is relevant as generally the contamination will be on the surface and not "in" the meat. One of the influencing factors for microbial growth, e.g., atmosphere, will be constant in this study (vacuum), therefore, it won't influence the different growth morphologies (immersed or surface colonies). Additionally, the cell concentration will be measured at a macroscopic level, therefore, the influence of the (model) food matrix wouldn't be visualised microscopically for its influence on the microbial growth. For these two reasons, only surface inoculation will be adequate for the experimental design of this study. Finally, the most appropriate protocol for the preparation of each model system will be investigated, in terms of raw materials, sterilisation method and experimental set-up.

Materials and methods

Model systems preparation

The protocol for the preparation of the model systems was developed by trial and error tests and the outcome is presented in the results section. An overview of the different conditions tested, in terms of the fat source, type of emulsifier and gelling agent is provided in Table 1. In this section, the general protocol for the preparation of different model systems is described. The composition is based on the chemical analysis of Frankfurter sausages, which has previously been characterised (Baka et al., 2015). Different sterilisation methods were also tried and documented in the results section, in order to select the most appropriate method. For the

preparation of the model systems, two aqueous solutions were prepared. The two different aqueous solutions were sterilised by using two different methods, based on the heat-sensitivity of their compounds. The 'solution 1', based on the heat-resistant components included NaCl, NaNO₂, pentasodium triphosphate, emulsifier, starch and agar, which were diluted in water and autoclaved. NaCl, NaNO₂ and pentasodium triphosphate, which are easily dissolved in water, were first added and stirred in cold water until they were dissolved. Then, the starch was added and stirred, while the temperature increased until it reached 80°C. Further on, for the emulsion and gelled emulsion systems, the emulsifier was added and stirred, until it became homogeneous, keeping the temperature constant at 80°C. Afterwards, the agar was added and stirred, until homogenisation at the same temperature (for the aqueous gel and gelled emulsion systems). The amount of water was 25.9, 25.4, 24.9 and 30.4 g for liquids, aqueous gels, emulsions and gelled emulsions, respectively. This amount of water is less than the total amount of water, which is provided in Table 2. The 'solution 2' included the heat-sensitive compounds, protein and ascorbic acid, which were diluted in water and filter sterilised, by using a 0.22 µm filter (Filtertop, TPP, Switzerland). The amount of water was 59, 59, 41 and 35 g for liquids, aqueous gels, emulsions and gelled emulsions, respectively. The latter amounts of water with the addition of the previously mentioned provide the sum of water used for each model system (Table 2). In this table, the ingredients which are presented were weighed in two different ways. In the first case (values with two decimals), the ingredients were directly weighed by using an analytical balance (CP3202P, Sartorius, Germany) of a two decimals precision. In the second case (values with four decimals), a concentrated aqueous solution of the ingredient was initially prepared which was further serially diluted in order to provide the concentration as presented in Table 2. Both solutions were mixed with a magnetic stirrer ('solution 3'), while the 'solution 1' was at 80°C. In the emulsions and gelled emulsions pork fat was also included. This was melted at 80°C in a water bath (GR150 S12; Grant, Shepreth, U.K.) and left at this temperature

for 2 h to eliminate the background microflora and keep it liquid. Fat was added to the ‘solution 3’ slowly, while the mixture is being homogenised with a homogenisation probe (S 18 N - 19 G, IKA, Belgium) attached to the homogeniser (Ultra-Turrax, IKA, Belgium) at 24000 rpm for 6 min (3x2min). Breaks were made every 2 min in order to avoid overheating of the sample and the disperser. Aqueous gels and gelled emulsions (7 mL) were dispersed in sterile petri dishes (Ø 50mm, height 8mm, SIMPD210-14, VWR, Belgium) (7 mL/petri dish). The canned meat was produced in a laboratory-scale production line of meat products of KU Leuven Technology Campus of Gent, Belgium. The meat tissue was blended and comminuted with fat and preservatives in a bowl cutter, in a similar way as meat slurries are being produced in the meat industry. Then, it was packaged in cans and autoclaved. The canned meat was sliced with thickness of < 4mm and then moulded by the round shape and size of small petri dishes, under sterile conditions.

Model systems characterisation

The model systems were characterised by determining their pH, water activity (a_w), and resistance to penetration, before cell inoculation. The pH was measured with a pH meter (DocuMeter, Sartorius, Germany), connected to two types of electrodes: (i) for liquid and emulsion systems, a pH electrode appropriate for homogeneous liquid systems (PY-P11, Sartorius, Germany) and (ii) for aqueous gel, gelled emulsion systems and canned meat, a pH electrode (HI 1413B, Hanna Instruments) appropriate for pH measurement on food surfaces. The water activity of all model systems was measured with an a_w -Kryometer (AWK-40, NAGY) on ‘liquid’-modus for liquid and emulsion systems and on ‘solid’-modus for aqueous gels, gelled emulsions and canned meat. Values of pH and water activity were determined in duplicate per model system. The resistance to penetration was determined with a texture analyser (TA.XTplus, Stable Micro Systems Ltd., Netherlands). The resistance to penetration was measured as the maximum force applied to the sample to achieve a depth of penetration of

2 mm, with a probe of 1 cm², with 0.1 mm/s penetration speed. Tests were conducted in duplicate for each storage temperature (4, 8 and 12°C) in order to evaluate the effect of temperature on the firmness of the model systems. Samples were placed in normal petri dishes with a thickness of 7.9 mm (50 mL of sample) and the probe targeted the centre of the petri dish.

Bacterial strains and preculture conditions

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, U.K.) supplemented with 25% (v/v) glycerol (Acros Organics, Belgium). For the inoculum preparation, a loopful of the stock culture was inoculated into a 100 mL Erlenmeyer flask containing 20 mL BHI, which was incubated in a temperature-controlled incubator at 30°C (Binder KB-series incubator; Binder Inc., NY, USA). After 22 h, 20 µL were transferred into 20 mL fresh BHI and again incubated at 30°C for 22 h.

Inoculation and culture conditions

A target inoculum level of 10² CFU/g of *L. monocytogenes* cells was inoculated in the final liquid and emulsion model systems and mixed at ambient temperature adequately until they become homogeneous. Then, 5 mL of the systems were transferred into vacuum bags of polyethylene/polyamine (PE/PA), vacuumed packed (Boxer 42, Henkelman, Netherlands) and incubated at 4, 8 and 12°C. The target inoculum level of *L. monocytogenes* for aqueous gels, gelled emulsions and canned meat was 10² CFU/g and was inoculated on the model systems at ambient temperature. Cell concentration was expressed in CFU/g also for the solid systems, in order to facilitate comparison among the different (micro)structures. Additionally, cells might migrate from the surface of the solid systems to the interior of them. Therefore, CFU/g is the most appropriate unit for expressing the cell concentration for all model systems. The cells aliquot was homogeneously spread throughout the whole surface of each model system with

polypropylene cell spreader (VWR, Belgium). Then, the Petri dishes were vacuum packaged separately, in vacuum bags and incubated at 4, 8 and 12°C. The selection of the storage temperature was made to simulate normal refrigeration temperatures (4°C) and abused temperatures (8 and 12°C). It is worth to comment that the range of the storage temperature covers a small region of suboptimal temperature for *L. monocytogenes* growth.

Microbiological analysis

Evolution of *L. monocytogenes* concentration over time in the different model systems was monitored by viable plate counting. For liquid and emulsion systems, the corresponding serial decimal dilutions were directly prepared from an aliquot of the samples and plated with a Spiral Plate System (Eddy Jet IUL Instruments, Belgium) on BHI supplemented with agar (1.4% (w/v); Agar Technical No3, Oxoid, U.K.). For aqueous gels, gelled emulsions and canned meat, prior to making serial decimal dilutions and sample plating, the content of a Petri-dish and a standard volume of peptone water (10 g/L bacteriological peptone (Oxoid, U.K.) and 5 g/L NaCl (AnalaR, Normapur®, VWR, Belgium)) were aseptically poured in a stomacher bag, and homogenised for 2 min (basic masticator, Led techno, Belgium). Plates were incubated at 30°C for 48 h before enumeration. Every experiment was independently performed in microbiological duplicates. All food (model) systems were checked for possible recontamination by plating and enumerating the first and the last sample in both BHI and Palcam agar plates. Palcam is a selective medium for *Listeria* species identification. If higher numbers in plate counts were observed on BHI agar plates, then possible re-contamination would have occurred.

Modelling and parameter estimation

To estimate the growth parameters of *L. monocytogenes*, the Baranyi and Roberts model (Baranyi and Roberts, 1994) was fitted to the experimental data. 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.). Standard errors of parameter estimates were calculated from the Jacobian matrix. A global estimation procedure was standardised for each model system. Additionally, $\mu_{\max}$ predictions of *L. monocytogenes* were obtained by the ComBase (<http://www.combase.cc/>), web-based open access database for microbial behaviour.

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. Standardised skewness and standardised kurtosis were used to assess if data sets came from normal distributions. These analyses were performed using Matlab version R2010b (The Mathworks Inc.). Test statistics were regarded as significant when p was $\leq (0.05 * \text{with the number of pairs to be compared})$, in order to avoid the Type I error (Bonferroni adjustment).

Results

Development of the protocol for the preparation of the model systems

In this study, the protocol for the preparation of the model systems was developed. Different sources of fat/oil, emulsifiers, and gelling agents were evaluated for providing some of the most complex systems with the targeting properties. Emulsifiers and gelling agents were also assessed for their optimal concentration. Among the pure pork fat, olive oil, corn oil and sunflower oil, the pork fat exhibited the best emulsification properties. It required the longest time for phase separation at room temperature, in comparison to the oils at the same concentration (18% (w/w)). Therefore, it was selected as the fat source for the emulsions and gelled emulsions. Three different types of emulsifiers appropriate for meat products (citrem 3302, 3307 and 3327) were tested at a 0.5% (w/w). Among them, the citrem 3302 created the

most long-lasting stable emulsions, where phase separation of aqueous and lipid phase did not occur, at room temperature storage. As this amount of emulsifier was not enough to keep emulsion stable for a long storage period, different concentrations of the citrem 3302 were tried, in order to create stable emulsions, where aqueous phase did not separate from lipid phase. The lowest concentration for stable emulsions at room temperature for one week was 1% (w/w). The most suitable gelling agent to prepare the aqueous gels and gelled emulsion systems was investigated. Gelatine, k-carrageenan, and agar were examined for their ability to solidify liquid and emulsion systems. Gelatine required a high concentration to solidify the medium, up to 10% (w/w). Gelatine is a protein and the used amount would change the composition of the model systems in protein, in the media to which it would be added. As the amount to be added would significantly change the total protein concentration, gelatine was excluded from further investigation. K-carrageenan at 0.5, 0.6, 0.8% (w/w) yielded very weak gels. However, at concentrations over 0.9% (w/w) they became solid very fast, although at high temperature, a fact that did not allow dispersing the whole mixture in Petri dishes. Consequently, k-carrageenan was not considered for further use. Agar was also tested at a concentration of 0.5% (w/w), which is easy to handle and provided good gelation properties to both aqueous gels and gelled emulsions. All these results are summarised in Table 1. The sterilisation method that was first assessed was to autoclave the model systems after their final mixture. This attempt was not successful for emulsions and gelled emulsions; the (micro)structure and the properties of these model systems altered after heat treatment. An alternative method was considered, which takes into account the proteins' degradation at high temperature and allows emulsification at low temperatures. All previously described tests led to the final recipe of the model systems. Their composition is provided in detail, in Table 2.

Physicochemical analysis of model systems

The average values of pH and water activity for the different model systems, at room temperature, are provided in Table 3. The values of pH and water activity slightly decrease while the structural complexity of the model systems increases. The highest average pH and water activity values are 6.07 and 0.9714 respectively, and belong to liquid systems, in contrast to the lowest which are 5.69 and 0.9460 and belong to gelled emulsion systems. An exception is observed for the water activity of canned meat, which is the highest of all model systems. The standard deviation among the measured values of pH and water activity is very small, which indicates the reproducibility of the model systems. The resistance to penetration was only relevant for the solid model systems (aqueous gels, gelled emulsions and canned meat) and is illustrated in Table 4. In this table, no obvious effect of the storage temperature was observed on the resistance to penetration for the three model systems. The canned meat was the firmest solid model system, although it exhibited the highest standard deviation among the solid model systems. Aqueous gels exhibited smaller resistance to penetration than canned meat, but higher than gelled emulsions. Both model systems, in contrast to the canned meat, had very small standard deviations from the average value of the resistance to penetration. This observation indicates the reproducibility of model systems, in terms of physicochemical characteristics, in contrast to real food products.

*Microbial dynamics of *L. monocytogenes* in variable (micro)structures*

L. monocytogenes growth dynamics in/on the different model systems are illustrated in Figure 2, at 4, 8 and 12°C. The model of Baranyi and Roberts (continuous lines) was fitted to the collected experimental data (dots). It is observed that *L. monocytogenes* grows faster on canned meat, then in liquid systems and on aqueous gels and finally, in emulsions and on gelled emulsions. Potential recontamination was assessed at all the food (model) systems, by plating and counting the first and the last sample on both BHI and Palcam agar plates. Results demonstrated equal counts on both culture media, indicating that no re-contamination occurred.

The estimated growth parameters of *L. monocytogenes* (maximum cell density, ' $N_{\max}$ ', maximum specific growth rate, ' $\mu_{\max}$ ' and lag phase, 'lag') for all the model systems are plotted for the three different storage temperatures in Figure 3. It was observed that $N_{\max}$ values obtained on canned meat are higher than the values obtained for the other model systems, at all temperatures. Among the model systems, the relation of the $N_{\max}$ values was: $N_{\max}$ of aqueous gels > $N_{\max}$ of liquids > $N_{\max}$ of gelled emulsions > $N_{\max}$ of emulsions. An exception can be noticed, at 4°C, where the $N_{\max}$ is higher in emulsions than on gelled emulsions. The highest $\mu_{\max}$ values of *L. monocytogenes* were obtained on canned meat at all temperatures. Lower $\mu_{\max}$ values were estimated on aqueous gels and in liquids, where higher $\mu_{\max}$ values were obtained in liquids at 4 and 8°C and on aqueous gels at 12°C. The lowest $\mu_{\max}$ values were estimated on gelled emulsions and in emulsions. Between them, higher $\mu_{\max}$ values were exhibited on gelled emulsions at 8 and 12°C, and vice versa at 4°C, at which there was no growth and therefore, the $\mu_{\max}$ on gelled emulsions was zero. The shortest lag values were estimated on canned meat products, at all temperatures. Longer lag values were obtained in liquids and on aqueous gel systems. Between both systems, shorter lag values were obtained on aqueous gels at 8 and 12°C, and vice versa at 4°C. The highest lag values were obtained in emulsion and on gelled emulsion systems. At 12°C, the lag estimates were shorter on gelled emulsions, at 8°C, in emulsions and at 4°C, lag value on gelled emulsions could be considered infinite, as no growth of *L. monocytogenes* occurred after 1000 h of sampling (Figure 2).

L. monocytogenes growth on canned meat versus Frankfurters

In this study, canned meat was produced in order to simulate Frankfurter sausages in composition, physicochemical and (micro)structural aspects. Therefore, it is of high importance to compare the growth dynamics of *L. monocytogenes* on the canned meat, and on real Frankfurter sausages, under the same storage conditions. The authors of this paper have already reported the growth dynamics of the same strain of *L. monocytogenes* in two types of

commercially available Frankfurters (Baka et al., 2015). In order to facilitate the comparison, growth parameters estimated in both studies are numerically provided in Table 5 and compositional analysis in Table 2. It can be clearly observed that the growth parameters of *L. monocytogenes* on canned meat approach those on Frankfurter (type 1), which also has more similar compositional aspects with canned meat. More specifically, $N_{\max}$ is higher on canned meat and lower on Frankfurters (type 2) at all temperatures; $N_{\max}$ estimate on canned meat is closer to $N_{\max}$ on Frankfurter type 1 than that of type 2. The $\mu_{\max}$ estimate of canned meat and Frankfurter (type 1) is identical at 4°C and smaller on the type 2 sausage. At 8 and 12°C, $\mu_{\max \text{ type1}} > \mu_{\max \text{ canned meat}} > \mu_{\max \text{ type2}}$, with $\mu_{\max \text{ canned meat}}$ estimate closer to the $\mu_{\max \text{ type1}}$. The highest lag phase estimate was on type 2 sausage and the lowest on canned meat at 4°C. At 8 and 12°C, the canned meat exhibited lag estimates > 0 , although on the other two sausage types was zero.

L. monocytogenes $\mu_{\max}$ estimates versus predictions of ComBase

In Figure 4, the $\mu_{\max}$ estimates of *L. monocytogenes* in/on the different model systems is compared with the ComBase predictions for *L. monocytogenes* in liquid microbiological media varying in storage temperature, pH and a_w , according to the measured values presented in Table 3 for the different meat-based (model) systems. Results indicate that there is a clear influence of these physicochemical characteristics on the predicted $\mu_{\max}$ of ComBase. Based on the predictions the relationship of the $\mu_{\max}$ would be as follows: $\mu_{\max}$ of liquids $>$ $\mu_{\max}$ of canned meat $>$ $\mu_{\max}$ of aqueous gels $>$ $\mu_{\max}$ of emulsions $>$ $\mu_{\max}$ of gelled emulsions. The estimates of $\mu_{\max}$ of *L. monocytogenes* in/on the meat-based (model) systems do not follow the same sequence, a fact that indicates that other factors influence the $\mu_{\max}$ values in/on these model systems. The other influencing factor which is not included in the ComBase predictions is the effect of the (micro)structure that varies among the different model systems. Nevertheless, a combination of all these factors define the $\mu_{\max}$ values of *L. monocytogenes*.

Discussion

The influence of temperature on growth parameters is evident for all model systems. In Figures 2 and 3, it can easily be seen that *L. monocytogenes* grows faster at higher temperature, as already reported in literature (Ratkowsky et al., 1982). It is noteworthy that the range of temperature considered in this study is very small and belongs to the suboptimal temperature for the growth of *L. monocytogenes*. Nevertheless, the key results of this study are the growth dynamics of *L. monocytogenes* in/on the different model systems and canned meat. The growth dynamics of *L. monocytogenes* are being compared and discussed based on two criteria. The first criterion concerns compositional factors and involves two groups. The first group includes the model systems without fat (liquids and aqueous gels). These have the highest compositional variation from the target food system (Frankfurters), due to the absence of fat that is replaced by water. The second group includes the food (model) systems, where fat is added and they approach in composition the target food systems (emulsions) or are compositionally identical (gelled emulsions and canned meat). The second criterion is the growth morphology, where *L. monocytogenes* growth is also separated in two groups. The first group includes colonial growth on the surface of solid food (model) systems (aqueous gel, gelled emulsion, canned meat) and the second group includes the planktonic growth in liquids and emulsions model systems. Schematic representation of these criteria and their different groups is provided in Table 1. The model systems are compared based on the previously mentioned criteria. Finally, results on canned meat will be compared with those in/on all the other model systems. This decision will facilitate the interpretation of the results and the indication of the most influential factors, on the growth of *L. monocytogenes* with respect to composition, physicochemical characteristics and structural characteristics, in which the model systems vary.

L. monocytogenes growth in liquids versus aqueous gels (criterion 1, group 1)

The differences in water activity are due to the addition of agar on the aqueous gel, which binds water and replaced an amount of it; therefore, the water activity of aqueous gel is lower than in

liquid systems. This variance in water activity could favour the growth of *L. monocytogenes* in liquid systems, where a_w was higher. Nevertheless, *L. monocytogenes* exhibited very similar growth dynamics in/on both systems (Figure 2), indicating that this level of variance in water activity does not affect *L. monocytogenes* growth. Another factor which could affect microbial growth between these two systems is the difference in growth morphology, planktonic versus surface colonial growth, which takes place in liquid systems and on the aqueous gels, respectively. In literature, it has been reported that microbial growth is affected by a solid environment, and specifically planktonic growth is faster than surface growth (Noriega et al., 2010b; Theys et al., 2008; Wilson et al., 2002). It has been documented that the decrease of growth on solid surfaces is due to diffusional limitations of nutrients (Antwi et al., 2006), and oxygen (Wimpenny and Coombs, 1983) and to the concentration gradients of metabolites formed around the colony, which induce a local pH drop (Malakar et al., 2003). However, in this study, *L. monocytogenes* growth between the two systems was similar, neglecting the influence of the growth morphology. Similar microbial dynamics in liquids and on aqueous gels have also been observed in literature (Smet et al., 2015). Nevertheless, growth parameters will be discussed one by one. N_{max} in liquid systems was lower than on aqueous gels at all temperatures, which is in accordance to what has been found by other studies (Robins and Wilson, 1994; Smet et al., 2015). In early stationary phase, bacteria influence their own environment, with their metabolites production, which inhibits growth (Robins and Wilson, 1994). In liquid systems, the transportation of these compounds is faster through convection than diffusion, which occurs on solid-like systems. Nevertheless, on solid surfaces when colonial morphology occurs, these compounds get in direct contact with the cells.

The μ_{max} estimates are higher in liquid systems at 4 and 8°C, but at 12°C is the opposite. This observation indicates that stressing temperature favours planktonic microbial growth, although at more optimal temperature levels microbial growth on solid surfaces is more favourable, as

also reported in Smet et al. (2015). It has been reported that $\mu_{\max}$ in liquid systems is higher than $\mu_{\max}$ on solid surfaces (Brocklehurst et al., 1997; Meldrum et al., 2003; Theys et al., 2008). Significant differences at $\mu_{\max}$ values between these two systems were induced when liquid systems were continuously shaken during incubation (Smet et al., 2015). When liquid cultures are shaken, nutrients, metabolites and oxygen are homogeneously transported and cell sedimentation is avoided, which also influences the distribution of the previously mentioned compounds (Smet et al., 2015). Therefore, when liquid systems are incubated without shaking exhibit negligible differences in $\mu_{\max}$ values or even lower than $\mu_{\max}$ values on solid surfaces. In this study, all model systems were vacuum packed; therefore, oxygen is not a variable in none of the model systems and has no influence on the results.

Lag phase of *L. monocytogenes* was longer, without statistically significant differences, in liquid systems at 8 and 12°C; however, at 4°C it was shorter, also without statistically significant differences. This is another indication of the effect of the storage temperature on the growth dynamics when compared within different (micro)structures. Likewise to the $\mu_{\max}$, stressing temperature favours microbial growth more in liquids (shorter lag) than on surface of aqueous gels. Nevertheless, no significant variation is usually observed between lag phase duration of cells growing in liquids or on solid surfaces, in literature (Boons et al., 2013b; Smet et al., 2015). Exceptionally, lag duration of cells growing on a gel surface was found longer than in planktonic growth at 25°C (Knudsen et al., 2012). Variation in composition among the used model systems in other studies and in this study could be the key for the existence or not of differences at the lag phase between planktonic and surface growth.

L. monocytogenes growth in emulsions versus gelled emulsions (criterion 1, group 2) Differences in growth dynamics in these two model systems are pronounced. Growth in emulsion systems with fat concentration lower than 83% is considered planktonic (Brocklehurst and Wilson, 2000). Therefore, as in the previous section, planktonic growth is compared with

surface growth. These two model systems differ from the previous two in the presence of fat and emulsifiers. The addition of fat increases the complexity in (micro)structure, as fat droplets are dispersed in the continuous water phase for emulsions and the continuous gellified water phase for gelled emulsion. It has been reported that cells grow in the aqueous phase (Wilson et al., 2002); therefore, the addition of fat decreases the amount of water available for the cells to grow. The $N_{\max}$ values of *L. monocytogenes* in emulsion systems were smaller than on gelled emulsions at 8 and 12°C. This observation is in agreement to the relation of $N_{\max}$ values between liquids and aqueous gels and generally to planktonic and surface growth (Robins and Wilson, 1994; Smet et al., 2015). However, $N_{\max}$ values in emulsions at 4°C were higher than on gelled emulsions. Therefore, an influence of temperature on $N_{\max}$ relation between the two model systems is evident. Specifically, the liquid environment at 4°C is more favourable than the solid environment or the other cells to which *L. monocytogenes* is in contact for its maximum cell density. Alternatively, it could refer to rheological instability of the model systems at variable storage temperatures. This specific aspect has partly been tested in this study, e.g., firmness assay. Results in Table 4 illustrated that there is variation of the measured resistance to penetration, referring to the firmness of the solid model systems, depending on the storage temperature. However, no specific trend of the influence of storage temperature on the firmness of the model systems has been observed.

The $\mu_{\max}$ values are statistically significant different and specifically, higher in emulsions than on gelled emulsions at 4°C and the opposite occurs at 8 and 12°C. This observation is in agreement with the relationship of $\mu_{\max}$ values in liquids (planktonic growth) and surfaces of solid systems, as previously discussed. When temperature becomes very stressing, it gets in favour of planktonic growth. This implies that the liquid environment which surrounds the cells at 4°C is more favourable for growth than the solid surface, or other cells with which the cells growing in colonies are in touch. The same observation was made for the $N_{\max}$, as well. Overall,

the addition of fat in either liquids or on solid systems does not influence the $\mu_{\max}$ relationship between the two growth morphologies, taking into account also the temperature factor. Nevertheless, fat affects the actual value of $\mu_{\max}$. This is later on discussed.

Finally, *L. monocytogenes* lag values in emulsion systems are significantly shorter than in gelled emulsion systems at 4 and 8°C. The opposite trend occurs at 12°C. This observation is in agreement to what has been discussed for the other growth parameters, about the influence of temperature on the growth of *L. monocytogenes* in systems of different (micro)structures and where different growth morphologies take place.

L. monocytogenes growth in liquids versus emulsions (criterion 2, group 1)

Comparison of *L. monocytogenes* growth dynamics between the systems where planktonic growth occurs, but the model systems vary in their structural complexity, is interesting for discussion. The differences in a_w measurements are due to the addition of fat and emulsifier; the emulsifier decreases the pH and fat which replaces an equal amount of water decreases the water activity. The relationship of *L. monocytogenes* growth parameters between those two systems is more straightforward. $N_{\max}$ and $\mu_{\max}$ are higher in liquids than emulsions and the reverse relationship is evident for the lag phase estimation at all temperatures. In literature, if the concentration of the fat in oil-in-water emulsions is less than 83% or 70% (Brocklehurst and Wilson, 2000; Kumar and Kumar, 2008), respectively, then microbial growth is similar to growth in liquid systems, respectively. In this study the fat concentration is limited to 18% (w/w). Therefore, *L. monocytogenes* growth should have been similar in liquids and emulsion systems. Although the morphology of growth is planktonic to both model systems, the growth dynamics of *L. monocytogenes* are not similar, but exhibit high differences. This can be due to the limitation of the aqueous (continuous) phase, which is replaced by the presence of fat and by the fact that preservatives are more condensed in aqueous phase of emulsions than in liquids. An exception is present for the $\mu_{\max}$ at 4°C, where in emulsions is higher than liquids. This

corresponds to a special situation, where the exponential phase was characterised by only a few data points, as growth was very slow. Growth, in this case, is described by a very long lag phase, and an exponential phase which exhibits a very fast switch from the moment it enters the exponential growth until the moment it enters the stationary phase. In fact, the exponential phase of growth might be an artefact of fitting the non-linear regression (the Baranyi and Roberts model) on the experimental data, as growth was less than 1.5 ln units (0.6 log₁₀ units). Therefore, the $\mu_{\max}$ is overestimated. For this reason, in Figure 4 the estimated $\mu_{\max}$ value for emulsions is set to zero.

L. monocytogenes growth on aqueous gels versus gelled emulsions (criterion 2, group 2) Those are two solid-state model systems, with and without the presence of fat with differences in water activity values, induced by the addition of fat and emulsifier in gelled emulsions. In this case, the differences of the growth parameters of *L. monocytogenes* are evident. The estimates of $N_{\max}$ and $\mu_{\max}$ on aqueous gels are higher than on gelled emulsions, while the lag duration is shorter at all temperatures. An exception takes place at 8°C, where the $N_{\max}$ of *L. monocytogenes* on gelled emulsions is higher than the one on aqueous gels. Those two model systems were analysed for their resistance to penetration; aqueous gels were firmer gels than gelled emulsions. It is noteworthy from Figure 2 and Table 4 that the systems with higher resistance to penetration were those on which *L. monocytogenes* grew faster. It is possible that firm surfaces allow only surface growth, however less firm might allow cell migration within the model (food) system. This hypothesis requires further investigation and specific quantification of the limits allowing cell penetration. Considering that on gelled emulsions (less firm model systems) cell penetration occurs, then both surface and immersed colonial growth takes place. It has recently been reported by Smet et al. (2015) that surface colonies grow faster than planktonic cells and the slowest growth is observed in immersed colonies, an observation which can justify the slowest growth dynamics for immersed colonies (gelled emulsion) than

for surface colonies (aqueous gel). In literature, the gelling agent concentration defining the firmness of the gel has an influence on the growth dynamics of immersed colonies. Firmer gels, of higher concentration of gelatine, inhibited the growth of *Listeria innocua*, in contrast to less firm gels of lower gelatine concentration (Antwi et al., 2006). In this study, the firmness of gels is affected by the combination of ingredients added in the model systems. Therefore, both composition and gelling agents' concentration play a decisive role on the influence on the growth dynamics of microorganisms.

L. monocytogenes growth on canned meat versus model systems and Frankfurters

L. monocytogenes exhibited the fastest growth, in terms of $N_{\max}$, $\mu_{\max}$ and lag, on canned meat products, than in/on any other model system, no matter the temperature. This finding is in contrast to what has been reported in literature. In Meldrum et al. (2003), Pin and Baranyi (1998) studies, it has been demonstrated that models developed based on experiments in liquid media overestimate microbial growth on real foods. This observation is confirmed by data provided in Figure 4, where Combase predictions in liquid media exhibit higher $\mu_{\max}$ values than those obtained by experimental data on solid surfaces (canned meat, aqueous gel and gelled emulsion). However, the growth dynamics of *L. monocytogenes* in/on liquid and aqueous gels was very similar to the growth dynamics on the canned meat. In contrast, the growth dynamics of *L. monocytogenes* on canned meat were very different than on gelled emulsion, which was the model system intended to approach in all aspects the canned meat. The gelled emulsion model system was targeted to become similar to the canned meat, in terms of composition, physicochemical characteristics and (micro)structure. With respect to a_w of the gelled emulsion, in comparison to the canned meat, obvious differences were present. These differences could be the main, but not the only reason of the differences in the growth dynamics of *L. monocytogenes*. The other possible reasons could be on the one hand, the different sources of proteins used in these two systems, i) hydrolysed protein in the gelled emulsions and ii) proteins

from meat tissue in canned meat. These two different sources of protein provide the systems with different structural properties, which might have an influence on the microbial growth dynamics. The main structural difference might be related to their emulsification ability. It has been reported that highly hydrolysed proteins tend to saturate the continuous phase of an emulsion rather than adhere to the water–oil interface (Lam and Nickerson, 2013). On the other hand, the difference in mixing between the model systems and the canned meat could explain the variations among the growth dynamics of *L. monocytogenes* on them. The canned meat is blended, however the model systems are homogenised, a fact that affects the distribution of nutrients, proteins and fat. Additionally, a relationship between the resistance to penetration of the systems and the growth dynamics of *L. monocytogenes* was found. Canned meat, which was the firmest among the solid systems, allowed the highest growth dynamics of *L. monocytogenes*. Finally, model systems were successfully developed, covering the full range of (micro)structural complexity, exhibiting inevitably compositional and physicochemical variability due to the addition of fat and gelling agents. Successful isolation of the effect of food (micro)structure would be of high interest, by keeping constant the physicochemical and compositional factors among the model systems. The presence (emulsions and gelled emulsions) or absence (liquids and aqueous gels) of fat depending on the food structural category cause, by definition, compositional and physicochemical variations. In this study, despite some limitations, an overall satisfactory approach was reached, which provides preliminary information about the effect of structural complexity, in/on model systems similar to Frankfurter sausages.

As it was presented in the result section, *L. monocytogenes* growth dynamics were similar to the Frankfurter sausage type 1 of the previous study (Baka et al., 2015). As it has been reported in the latter study, the growth of *L. monocytogenes* was faster on the skinless sausage type 1. Sausage type 2 was surrounded by a casing that inhibited the growth of *L. monocytogenes*. In

this study, the canned meat was prepared without sausage casing, which isolates the meat product from the inoculated *L. monocytogenes* cells. Therefore, the real food system prepared in this study was validated to have similar *L. monocytogenes* growth dynamics as the real Frankfurter of similar composition.

L. monocytogenes $\mu_{\max}$ prediction of ComBase versus $\mu_{\max}$ estimation in/on meat-type (model) systems

The $\mu_{\max}$ estimates of *L. monocytogenes* obtained in/on the meat-based (model) systems were compared to the ComBase prediction for *L. monocytogenes* at the three storage temperatures (4, 8 and 12°C) and at pH and a_w equal to those measured on the different model systems, as presented in Table 3. By comparing the predictions with the estimates (Figure 4), it is conclusive that there are other factors than just the physicochemical variations among the model systems that influence the $\mu_{\max}$ of *L. monocytogenes* in/on the different meat-based (model) systems. This conclusion is drawn by the fact that the trend of the $\mu_{\max}$ among the different model systems based on the ComBase predictions is not the same as the estimates based on the experimental data. The predictions do not incorporate the influence of the (micro)structure of the different meat-based (model) systems, however, the $\mu_{\max}$ estimates consider this influencing factor. It is clear that the physicochemical variability among the model systems has an influence on the $\mu_{\max}$ estimates, but is not the only reason for these variations. The bias factor of the $\mu_{\max}$ predicted by ComBase and estimated by the experimental data, as provided in Mejlholm et al., 2010 was also calculated. The bias factor was found to be 1.77, which is a not acceptable value for the model to predict the experimental data, as it exceeds the 1.43 limit of acceptability for model predictions (Ross, 1999).

Conclusion

Significant differences among the estimated growth dynamics of *L. monocytogenes* have been found among those model systems that intentionally vary in (micro)structural complexity, and

inevitably in compositional and physicochemical characteristics. The difference in growth dynamics have been explained on the basis of one or a combination of factors: (i) composition, (ii) physicochemical properties, (iii) (micro)structure, (iv) temperature, (v) firmness and (vi) growth morphology. In this study, *L. monocytogenes* grew faster on the canned meat, which is a complex system regarding its structural properties and represents a real food system. Therefore, microbial growth on real foods can be faster than in/on model systems, which are frequently used to control and predict microbial dynamics and ensure microbiological food safety. Additionally, the often-reported fail-safe predictions from liquid systems, which overestimate growth on real foods, have not been confirmed in this work. Therefore, attention should be paid when using model systems, even when approaching real food products in all aspects and validation on real foods is always recommended to ensure the food microbiological safety.

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References

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- Antwi, M., Bernaerts, K., Van Impe, J.F. and 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 coculture. International Journal of Food Microbiology, 120: 71-84.
- Antwi, M., Geeraerd, A.H., Vereecken, K.M., Jenné, R., Bernaerts, K. and Van Impe, J.F. (2006). Influence of a gel microstructure as modified by gelatin concentration on *Listeria innocua* growth. Innovative Food Science and Emerging Technologies, 7: 124–131.
- Baka, M., Noriega, E., Mertens, L., Van Derlinden, E. and Van Impe, J.F. (2014). Protective role of indigenous *Leuconostoc carnosum* against *Listeria monocytogenes* on vacuum packed Frankfurter sausages at suboptimal temperatures. Food Research International, 66: 197-206.
- Baka, M., Noriega, E., Tsakali, E. and Van Impe, J. (2015). Influence of composition and processing of Frankfurter sausages on the growth dynamics of *Listeria monocytogenes* under vacuum. Food Research International, 70: 94-100.
- Baranyi, J. and Roberts, T.A. (1994). A dynamic approach to predicting bacterial growth in food. International Journal of Food Microbiology, 23: 277-294.
- Boons, K., Mertens, L., Van Derlinden, E., David, C.C., Hofkens, J. and Van Impe, J.F. (2013a). Behavior of *Escherichia coli* in a heterogeneous gelatin-dextran mixture. Applied and Environmental Microbiology, 9(9): 3126-3128.
- Boons, K., Noriega, E., Van den Broeck, R., David, C.C., Hofkens, J. and Van Impe, J.F. (2014). Effect of microstructure on population growth parameters of *Escherichia coli* in gelatin-dextran systems. Applied and Environmental Microbiology, 80(17): 5330-5339.
- Boons, K., Van Derlinden, E., Mertens, L., Peeters, V. and Van Impe, J.F. (2013b). Effect of immobilization and salt concentration on the growth dynamics of *Escherichia coli* K12 and *Salmonella* Typhimurium. Journal of Food Science, 78: 567–574.

- Brocklehurst, T.F., Mitchell, G.A. and Smith, A.C. (1997). A model experimental gel surface for the growth of bacteria on foods. *Food Microbiology*, 14: 303–311.
- Brocklehurst, T.F., Parker, M.L., Gunning, P.A., Coleman, H.P. and Robins, M.M. (1995). Growth of food-borne pathogenic bacteria in oil-in-water emulsions: II. Effect of emulsion structure on growth parameters and form of growth. *Journal of Applied Bacteriology*, 78: 609–615.
- Brocklehurst, T.F., Parker, M.L., Gunning, P.A. and Robins, M.M. (1993). Microbiology of food emulsions: physicochemical aspects. *Lipid Technology*, 83-88.
- Brocklehurst, T.F. and Wilson, P.D.G. (2000). The role of lipids in controlling microbial growth. *Grasas y aceites*, 51: 66-73.
- Castro, M.P., Rojas, A.M., Campos, C.A. and Gerschenson, L.N. (2009). Effect of preservatives, tween 20, oil content and emulsion structure on the survival of *Lactobacillus fructivorans* in model salad dressings. *Food Science and Technology*, 42: 1428-1434.
- Dervakos, G.A. and Webb, C. (1991). On the merits of viable-cell immobilisation. *Biotechnology Advances*, 9: 559-612.
- 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.
- EFSA/ECDC. (2015). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. *EFSA Journal* 2015, 13 (1): 3991.
- Farber, J.M., McKellar, R.C. and Ross, W.H. (1995). Modelling the effects of various parameters on the growth of *Listeria monocytogenes* on liver pâté. *Food Microbiology*, 12: 447– 453.
- Jeanson, S., Chadoeuf, J., Madec, M.N., Aly, S., Floury, J., Brocklehurst, T.F. and Lortal, S. (2011). Spatial distribution of bacterial colonies in a model cheese. *Applied and Environmental Microbiology*, 77: 1493–1500.

- Knudsen, G.M., Nielsen, M., Grassby, T., Danini-Appleton, V., Thomsen, L.E., Colquhoun, I.J., Brocklehurst, T.F., Olsen, J.E. and Hinton, J.C.D. (2012). A third mode of surface-associated growth: immobilization of *Salmonella enterica* serovar Typhimurium modulates the RpoS-directed transcriptional programme. *Environmental Microbiology*, 14: 1855–1875.
- Koutsoumanis, K.P., Kendall, P.A. and Sofos, J.N. (2004). A comparative study on growth limits of *Listeria monocytogenes* as affected by temperature, pH and a_w when grown in suspension or on a solid surface. *Food Microbiology*, 21: 415-422.
- Kumar, H.D. and Kumar, S. (2008). Microbial growth and food structure, p. 47-49. *In* Modern concepts of microbiology. VIKAS Publishing House PVT LTD, New Delhi.
- Lebert, I., Dussap, C.G. and Lebert, A. (2004). Effect of a_w , controlled by the addition of solutes or by water content, on the growth of *Listeria innocua* in broth and in gelatine model. *International Journal of Food Microbiology*, 94: 67-78.
- Malakar, P., Barker, G., Zwietering, M. and van't Riet, K. (2003). Relevance of microbial interactions to predictive microbiology. *Int. Journal of Food Microbiology*, 84: 263-272.
- Malakar, P.K., Brocklehurst, T.F., Mackie, A.R., Wilson, P.D.G., Zwietering, M.H. and van't Riet, K. (2000). Microgradients in bacterial colonies: use of fluorescence ratio imaging, a non-invasive technique. *International Journal of Food Microbiology*, 56: 71–80.
- Mebatsion, H.K., Verboven, P., Ho, Q.T., Verlinden, B.E. and Nicolai, B.M. (2008). Modelling fruit (micro)structures, why and how? *Trends in Food Science and Technology*, 19: 59-66.
- Mejlholm, O., Gunvig, A., Borggaard, C., Blom-Hanssen, J., Mellefont, L., Ross, T., Leroi, F., Else, T., Visser, D., Dalgaard, P. (2010). Predicting growth rates and growth boundary of *Listeria monocytogenes* - An international validation study with focus on processed and ready-to-eat meat and seafood. *International Journal of Food Microbiology*, 141: 137-150.

- Meldrum, R., Brocklehurst, T.F., Wilson, D. and Wilson, P. (2003). The effects of cell immobilization, pH and sucrose on the growth of *Listeria monocytogenes* Scott A at 10°C. Food Microbiology, 20: 97–103.
- Mertens, L., Van Derlinden, E., Dang, T.D.T., Cappuyns, A.M., Vermeulen, A., Debevere, J., Moldenaers, P., Devlieghere, F., Geeraerd, A.H. and Van Impe, J.F. (2011). On the critical evaluation of growth/no growth assessment of *Zygosaccharomyces bailii* with optical density measurements: liquid versus structured media. Food Microbiology, 28: 736–745.
- Noriega, E., Laca, A. and Díaz, M. (2008a). Modelling of diffusion-limited growth to predict *Listeria* distribution in structure model foods. Journal of Food Engineering, 87: 247-256.
- Noriega, E., Laca, A. and Díaz, M. (2008b). Modelling of diffusion-limited growth for food safety in simulated cheeses. Food Bioproducts Processing, 86: 122-129.
- Noriega, E., Laca, A. and Díaz, M. (2009). *Listeria* growth under diffusional limitations in synthetic meats. International Journal of Food Science and Technology, 44: 725-734.
- Noriega, E., Laca, A. and Díaz, M. (2010a). Development of a structure-based model for the competitive growth of *Listeria innocua* in minced chicken breasts. International Journal of Food Microbiology, 142: 44–52.
- Noriega, E., Laca, A., Diaz, M. (2010b). Decisive role of structure in food microbial colonization and implications for predictive microbiology. Journal of Food Protection, 73: 938-951.
- Parker, M.L., Brocklehurst, T.F., Gunning, P.A., Coleman, H.P. and Robins, M.M. (1995). Growth of foodborne pathogenic bacteria in oil-in-water emulsions: I – methods for investigating the form of growth. Journal of Applied Bacteriology, 78: 601-608.
- Pin, C. and Baranyi, J. (1998). Predictive models as means to quantify the interactions of spoilage organisms. International Journal of Food Microbiology, 41: 59-72.

- Ratkowsky, D.A., Olley, J., McMeekin, T.A. and Ball, A. (1982). Relationship between temperature and growth rate of bacterial cultures. *Journal of Bacteriology*, 149: 1–5.
- Lam, R.S.H. and Nickerson, M.T. (2013). Food proteins: A review on their emulsifying properties using a structure–function approach. *Food Chemistry*, 141(2): 975-984.
- Robins, M.M. and Wilson, P.D.G. (1994). Food structure and microbial growth. *Trends in Food Science and Technology*, 5: 289–293.
- Ross, T. (1999). Predictive Food Microbiology Models in the Meat Industry, pp. 196. *In Meat and Livestock Australia*. North Sydney, Australia.
- Smet, C., Van Derlinden, E., Mertens, L., Noriega, E. and Van Impe, J.F. (2015). Effect of cell immobilization on the growth dynamics of *Salmonella* Typhimurium and *Escherichia coli* at suboptimal temperatures. *International Journal of Food Microbiology*, 208: 75–83.
- Theys, T.E., Geeraerd, A.H., Verhulst, A., Poot, K., Van Bree, I., Devlieghere, F., Moldenaers, P., Wilson, D., Brocklehurst, T. and Van Impe, J.F. (2008). Effect of pH, water activity and gel micro-structure, including oxygen profiles and rheological characterization, on the growth kinetics of *Salmonella* Typhimurium. *International Journal of Food Microbiology*, 128: 67–77.
- Walker, S., Brocklehurst, T.F. and Wimpenny, J. (1997). The effects of growth dynamics upon pH gradient formation within and around subsurface colonies of *Salmonella typhimurium*. *Journal of Applied Microbiology*, 82: 610-614.
- Wareing, P. and Komitopoulou, E. (2013). Predictive modelling: Principles and Practice, pp. 203-228. *In* Taormina PJ (ed), *Microbiological Research and Development for the Food Industry*. CRC Press, Florida, FL.
- Wilson, P., Brocklehurst, T., Arino, S., Thuault, D., Jakobsen, M., Lange, M., Farkas, J., Wimpenny, J. and Van Impe, J.F. (2002). Modelling microbial growth in structured foods: towards a unified approach. *International Journal of Food Microbiology*, 73: 275–289.

Wimpenny, J.W.T. and Coombs, J.P. (1983). Penetration of oxygen into bacterial colonies.
Journal of Genetic Microbiology, 129: 1239–1242.

Wimpenny, J., Leistner, L., Thomas, L., Mitchell, A., Katsaras, K. and Peetz, P. (1995).
Submerged bacterial colonies within food and model systems: their growth, distribution and
interactions. International Journal of Food Microbiology, 28: 299-315.

Figure Captions

Figure 1. Demonstration of the model systems and schematic representation of the different criteria influencing microbial growth.

Figure 2. Growth dynamics of *Listeria monocytogenes* in/on liquids, aqueous gels, emulsions, gelled emulsions and canned meat systems at 4 (a), 8 (b) and 12°C (c). The experimental data of the liquids are represented with ( ) , aqueous gels with ( ) , emulsions with ( ) , gelled emulsions with ( ) and canned meat with ( ) . The Baranyi and Roberts model fit is depicted by the full line.

Figure 3. Growth parameters of *L. monocytogenes* (a) $\ln(N_{\max})$, (b) $\mu_{\max}$ and (c) lag, and their standard error (error bars) for the different model systems at 4 ( ) , 8 ( ) and 12°C ( ) . For each growth parameter (graphs a, b, c) and temperature (different colours and symbols), values bearing different characters are significantly different ($P \leq 0.05$).

Figure 4. *L. monocytogenes* $\mu_{\max}$ values as predicted by ComBase for the three storage temperatures 4°C ( ) , 8°C ( ) and 12°C ( ) at pH and a_w values, as measured on the different model systems and presented in Table 3. Additionally, *L. monocytogenes* $\mu_{\max}$ estimates as calculated by experimental data in/on the different model systems are represented with ( ) , ( ) , ( ) , for the three storage temperatures 4, 8 and 12°C, respectively.

Tables

Table 1. Source of fat, emulsifier, gelling agents tested in order to develop the model systems.

Table 2. Composition of model systems.

Table 3. pH and a_w of the model systems and canned meat and their standard deviation (SD).

Table 4. Resistance to penetration (N/cm^2) and the standard deviation of aqueous gels, gelled emulsions and canned meats at 4, 8 and 12°C.

Table 5. *L. monocytogenes* growth parameters on canned meat sausages and on Frankfurter sausages.

Figure 1

Figure 2

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Figure 3

Figure 4

Table 1. Source of fat, emulsifier, gelling agents tested in order to optimise the model systems.

	Compound	Specifications of compound	Concentration [% (w/w)]	Comments	Result
Source of fat	Pork fat	Local butcher	18.0	Phase separation < 24 h	OK
	Sunflower oil	Supermarket Delhaize	18.0	Phase separation < 2 h	X
	Olive oil	Supermarket Delhaize	18.0	Phase separation < 2 h	X
	Corn oil	Supermarket Delhaize	18.0	Phase separation < 2 h	X
Emulsifier	Emulsifier 1	Palsgaard Citrem 3302	0.5 / 1.0	Phase separation < 24 h / No phase separation >1 week at 20°C	OK
	Emulsifier 2	Palsgaard Citrem 3307	0.5	Phase separation < 8 h	X
	Emulsifier 3	Palsgaard Citrem 3327	0.5	Phase separation < 2 h	X
Gelling agent	Gelatine	Oxoid, UK	10.0	Very high concentration for firm gels	X
	K-carrageenan	Sigma Aldrich, Germany	0.5-1.0	Not practical results	X
	Agar	Agar technical (No.3), Oxoid, UK	0.5	Firm gels	OK

Table 2. Composition of model systems.

Ingredients (% w/w)	Description of Ingredients	Liquid	Aqueous gel	Emulsion	Gelled emulsion	Canned meat	Frankfurter type 1 **	Frankfurter type 2 **
Water	Distilled water	84.90	84.40	65.90	65.40	65.40	65.50	66.90
Protein	Primatone, Sigma Aldrich, Germany	12.00	12.00	12.00	12.00	12.00*	12.59	10.86
Fat	Animal fat (local butcher)	0.00	0.00	18.00	18.00	18.00	19.02	14.46
Starch	Soluble Powder, Fisher Scientific, UK	1.00	1.00	1.00	1.00	1.00	1.04	0.85
Agar	Agar technical (No.3), Oxoid, UK	0.00	0.50	0.00	0.50	0.50	--	--
Emulsifier	Citrem 3302, Paalgaard, Denmark	0.00	0.00	1.00	1.00	1.00	--	--
Sodium Chloride	Analar, Normapur, Belgium	2.00	2.00	2.00	2.00	2.00	2.18	1.91
Sodium Nitrite	Sigma Aldrich, Germany	0.0011	0.0011	0.0011	0.0011	0.0011	0.0008	0.0014
Ascorbic acid	Duchefa Biochemie, Netherlands	0.0056	0.0056	0.0056	0.0056	0.0056	0.0049	0.0084
Pentasodium Triphosphate	Sigma Aldrich, Germany	0.1000	0.1000	0.1000	0.1000	0.1000	0.0000	0.0956

*Protein from pork and beef in mixture ratio: 80/20, Geha meat.

**Measurements of Frankfurters' composition published in Baka et al., 2015.

Table 3. pH and a_w of the model systems and canned meat and their standard deviation (SD).

Systems	pH $\pm$ SD(pH)	$a_w\pm$ SD(a_w)
Liquid	6.07 $\pm$ 0.05	0.9714 $\pm$ 0.0016
Aqueous gel	5.91 $\pm$ 0.30	0.9561 $\pm$ 0.0044
Emulsion	5.88 $\pm$ 0.03	0.9511 $\pm$ 0.0012
Gelled emulsion	5.69 $\pm$ 0.01	0.9460 $\pm$ 0.0030
Canned meat	5.58 $\pm$ 0.03	0.9770 $\pm$ 0.0040

Table 4. Resistance to penetration (N/cm^2) and the standard deviation of aqueous gels, gelled emulsions and canned meats at 4, 8 and 12°C.

Model system	Temperature	4°C	8°C	12°C
Aqueous gels	Resistance to	0.471 ± 0.025	0.539 ± 0.045	0.503 ± 0.081
Gelled emulsion	penetration (N/cm^2) $\pm$	0.305 ± 0.006	0.277 ± 0.009	0.279 ± 0.021
Canned meat	Standard deviation	4.64 ± 0.43	4.78 ± 0.12	4.42 ± 0.41

Table 5. *L. monocytogenes* growth parameters on canned meat sausages and on Frankfurter sausages.

Temperature	Growth parameter	Canned meat	Frankfurter 1	Frankfurter 2
4°C	$\ln(N_{\max}) \pm SE(\ln(N_{\max}))$			
	[ln(CFU/g)]	20.75 ± 0.17^a	18.85 ± 0.27^b	17.79 ± 1.79^b
	$\mu_{\max} \pm SE(\mu_{\max})$ [1/h]	0.0301 ± 0.0006^a	0.0326 ± 0.0012^b	0.0182 ± 0.0009^c
	lag±SE(lag) [h]	33.5 ± 8.2^a	36.3 ± 14.9^a	50.2 ± 40.9^a
8°C	$\ln(N_{\max}) \pm SE(\ln(N_{\max}))$			
	[ln(CFU/g)]	20.58 ± 0.18^a	20.17 ± 0.20^a	19.14 ± 0.23^b
	$\mu_{\max} \pm SE(\mu_{\max})$ [1/h]	0.0530 ± 0.0012^a	0.0643 ± 0.0028^b	0.0422 ± 0.0015^c
	lag±SE(lag) [h]	7.15 ± 6.62^a	0.00 ± 0.00^b	0.00 ± 0.00^b
12°C	$\ln(N_{\max}) \pm SE(\ln(N_{\max}))$			
	[ln(CFU/g)]	21.21 ± 0.16^a	20.18 ± 0.08^b	19.11 ± 0.26^c
	$\mu_{\max} \pm SE(\mu_{\max})$ [1/h]	0.0940 ± 0.0027^a	0.1248 ± 0.0049^b	0.0534 ± 0.0025^c
	lag±SE(lag) [h]	2.32 ± 4.00^a	0.00 ± 4.45^a	0.00 ± 0.00^a

For each growth parameter, values bearing different superscripts (no characters in common) horizontally are significantly different (P≤0.05)