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

“*Salmonella Typhimurium* and *Staphylococcus aureus* dynamics in/on variable (micro)structures of fish-based model systems at suboptimal temperatures”

by Maria Baka, Davy Verheyen, Nicolas Cornette, Stijn Vercruyssen, Jan F. Van Impe

which has been archived on the university repository Lirias (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

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

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

M. Baka, D. Verheyen, N. Cornette S. Vercruyssen, J. F. Van Impe (2017). Salmonella Typhimurium and Staphylococcus aureus dynamics in/on variable (micro)structures of fish-based model systems at suboptimal temperatures. International Journal of Food Microbiology, 240, 32-39.

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

<https://www.sciencedirect.com/science/journal/01681605/240>

<https://www.sciencedirect.com/science/article/pii/S016816051630397X>

The corresponding author can be contacted for additional info.

Conditions for open access are available at:

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

***Salmonella* Typhimurium and *Staphylococcus aureus* dynamics in/on variable
(micro)structures of fish-based model systems at suboptimal temperatures**

Maria Baka^{a,b}, Davy Verheyen^{a,b}, Nicolas Cornette^{a,b}, Stijn Vercruyssen^{a,b}, Jan F. Van Impe^{a,b,*}

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

^bBioTeC – Chemical and Biochemical Process Technology and Control, KU Leuven

[[maria.baka](mailto:maria.baka@cit.kuleuven.be), [jan.vanimpe](mailto:jan.vanimpe@cit.kuleuven.be)][@cit.kuleuven.be](mailto:jan.vanimpe@cit.kuleuven.be).

***Author for correspondence:**

Jan F. M. Van Impe

BioTeC – Chemical and Biochemical Process Technology and Control

Department of Chemical Engineering, KU Leuven

Tel: +32 16 32 14 66

Fax: +32 16 32 29 91

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

ABSTRACT

The limited knowledge concerning the influence of food (micro)structure on microbial dynamics decreases the accuracy of the developed predictive models, as most studies have mainly been based on experimental data obtained in liquid microbiological media or in/on real foods. The use of model systems has a great potential when studying this complex factor. Apart from the variability in (micro)structural properties, model systems vary in compositional aspects, as a consequence of their (micro)structural variation. In this study, different experimental food model systems, with compositional and physicochemical properties similar to fish patés, are developed to study the influence of food (micro)structure on microbial dynamics. The microbiological safety of fish products is of major importance given the numerous cases of salmonellosis and infections attributed to staphylococcus toxins. The model systems under study represent food (micro)structures of liquids, aqueous gels, emulsions and gelled emulsions. The growth/inactivation dynamics and a modelling approach of combined growth and inactivation of *Salmonella* Typhimurium and *Staphylococcus aureus*, related to fish products, are investigated in/on these model systems at temperatures relevant to fish products' common storage (4°C) and to abuse storage temperatures (8 and 12°C). ComBase (<http://www.combase.cc/>) predictions compared with the maximum specific growth rate (μ_{max}) values estimated by the Baranyi and Roberts model in the current study indicated that the (micro)structure influences the microbial dynamics. Overall, ComBase overestimated microbial growth at the same pH, a_w and storage temperature. Finally, the storage temperature had also an influence on how much each model system affected the microbial dynamics.

Keywords: structure, microbial growth, microbial inactivation

1. Introduction

Predictive microbiology is a discipline of food microbiology in which accurate and versatile mathematical models are developed in order to describe microbial dynamics as a function of environmental conditions (Van Impe et al., 2005). The use of cumulative databases is a substantial advantage of predictive microbiology, as new data are collected regularly (McDonald and Sun, 1999; Ross and McMeekin, 1994). Since well-developed predictive models allow a realistic estimation of microbial dynamics in foods, these are a positive asset to food safety (Koutsoumanis et al., 2004). However, the limited knowledge concerning the influence of food (micro)structure on microbial dynamics reduces the accuracy of predictive models, as most studies have mainly been based on experimental data obtained in liquid microbiological media (Mertens et al., 2011). Food model systems, which simulate the properties of real foods, are essential to study the influence of food (micro)structure on microbial dynamics. The possibility to alter different factors independently from one another, the absence of interfering background microflora, and simple transferability of findings to other food products are the main advantages of using food model systems instead of real food products for these studies (Baka et al., 2016).

Fish products are important consumption products in Europe: approximately 8.8 million tonnes in 2015 (EUMOFA, 2015). Fish is necessary to a healthy human diet, as it provides high-quality proteins and essential omega-3 polyunsaturated fatty acids, which have demonstrated to be protective against a plethora of diseases, e.g., coronary heart disease, diabetes, hypertension, cancer, autoimmune disorders (Simopoulos, 1996; Vaclavik and Christian, 2014). However, fish products are also a common cause of foodborne diseases. In 2014, fish

products were the food vehicle in 6.8% of all foodborne outbreaks in Europe (EFSA and ECDC, 2015).

S. Typhimurium and *S. aureus* are two foodborne pathogens relevant to fish products (Mohamed Hatha, 1997; Rahimi et al., 2013; Saito et al., 2011). *S. Typhimurium* causes salmonellosis, with symptoms ranging from self-limiting gastroenteritis to life-threatening enteric fever (Cooke et al., 2007). *S. aureus* causes food poisoning due to the production of enterotoxins in food products (EFSA and ECDC, 2015; Saito et al., 2011). Between 1988 and 2007, 46.9% of all foodborne outbreaks were caused by *Salmonella*. In 1.7% of these *Salmonella*-related outbreaks, seafood products were the causative food vehicle. Similarly, 4.5% of all foodborne outbreaks were caused by *S. aureus*, with seafood products being the food vehicle in 3.3% of those outbreaks (Greig and Ravel, 2009).

In the present study, various experimental food model systems, simulating compositional and physicochemical properties of processed fish products (fish patés), are developed to study the influence of food (micro)structure on microbial dynamics. These model systems cover the entire range of possible food (micro)structures, i.e., liquids, aqueous gels, emulsions, gelled emulsions. Subsequently, the growth/inactivation dynamics of *S. Typhimurium* and *S. aureus* are investigated in and on these model systems at temperatures relevant for fish product storage (4°C) and abuse storage temperatures, occurring during transportation or by malfunctioning refrigerators (8 and 12°C).

2. Materials and methods

2.1. Model system development

For the gelled emulsion model system, the compositional and (micro)structural properties of fish patés were targeted. The composition of the gelled emulsion model system was established according to relevant literature (Aquerreta et al., 2002; Echarte et al., 2004; Gonçalves and Ribeiro Duarte, 2008; Tahergorabi et al., 2012). The other model systems (i.e., liquid, emulsion, aqueous gel), were developed based on the composition of the gelled emulsion, taking into account the specific characteristics of each (micro)structure (e.g., absence of fat and emulsifiers from the liquids and aqueous gels, absence of gelling agents from the liquids and emulsions). The model systems varied in physicochemical and compositional aspects, which is a consequence of achieving different (micro)structures. The composition of the model systems is provided in Table 1.

2.2. Model systems characterisation

The model systems were characterised by determining their pH and water activity (a_w) before cell inoculation. The pH was measured with a pH meter (DocuMeter, Sartorius, Germany), connected with 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 and gelled emulsion systems, 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 and gelled emulsions. The measured values are provided in Table 1.

2.3. Microorganisms and preculture conditions

S. aureus strain LMG 08195 was provided by the Belgian Co-ordinated Collection of Microorganisms of the Laboratory of Microbiology of the University of Gent (BCCM/LMG, Ghent, Belgium). *Salmonella enterica* serovar Typhimurium strain SL1344 was provided by the Institute of Food Research (IFR, Norwich, UK).

Stock cultures were stored at -80°C in Brain Heart Infusion (BHI, Oxoid, Hants, UK) supplemented with 20% (v/v) glycerol (Glycerol Bidistilled 99.5%, VWR International, Leuven, Belgium). Inocula were prepared by transferring a loopful (10 µL) of the stock culture into 20 mL of BHI. After incubating for 22 h at 37°C under static conditions (Binder KB-series incubator; Binder Inc., NY, USA), 20 µL of the stationary-phase culture was inoculated into 20 mL of fresh BHI and incubated for 22 h under the same conditions. This resulted in stationary-phase cultures with an inoculum level of approximately 10^9 CFU/mL, as measured by plate counting. The preculture of each microorganism was prepared individually.

2.4. Challenge testing

The respective stationary phase preculture of *S. Typhimurium* and *S. aureus* was individually diluted to the appropriate cell level using peptone water, containing 1.8% w/v peptone (Peptone Bacteriological, Oxoid, Hants, UK) and 1.0% w/v NaCl. The different model systems were then inoculated to a final cell concentration of approximately 10^2 CFU/g. Each microorganism was grown individually in/on the model systems. Cell concentration is expressed in CFU/g for both types of inoculation (surface and homogeneous), in order to facilitate comparison (Baka et al., 2016). In liquid and emulsion model systems, a cell aliquot was inoculated (in the Schott bottles) and bottles were shaken to obtain a homogeneous dispersion of viable cells. For aqueous gel and gelled emulsion model systems, inocula were transferred onto the surface of each model system (in the petri plates) and spread using a disposable L-shaped cell spreader. Afterwards, the different inoculated model systems were incubated at temperatures of 4, 8 and

12°C (Termaks inc., Bergen, Norway). Samples were taken at different time intervals. For model systems inoculated with *S. Typhimurium*, the maximum sampling time was 1200 h (50 d), with 10-16 sampling points per model system for the experiments at storage temperature of 4°C, and 600 h (25 d), with 11-20 sampling points for the experiments at temperatures of 8 and 12°C. For model systems inoculated with *S. aureus*, the maximum sampling time was 500 h ($\approx$ 21 d), with 7-10 sampling points for the experiments at temperatures of 4 and 8°C, and 800 h ($\approx$ 33 d), with 14-20 sampling points for the experiments at storage temperature of 12°C. The duration of sampling varied among the model systems and storage temperatures. In most cases the target was to obtain the full growth curve (for growth experiments) or to observe microbial counts up to the detection limit, for inactivation or survival curves (10 CFU/mL) (Sutton, 2011). For the liquid and emulsion model systems, a sample of 400 μ L was taken from the respective Schott bottles at each time interval, after shaking the bottles to homogenise the sample. For the aqueous gels and gelled emulsions, the content of the petri dishes was transferred into a sterile stomacher bag to which a known volume of peptone water was added. In order to detach the microbial cells from the gels and disperse them into the solution, the bag was then placed into a stomacher (Stomacher, IUL, Barcelona, Spain) for 30 s. For all model systems, samples were decimally diluted using peptone water, and plated onto BHI agar plates, containing 3.7% w/v BHI and 1.4% w/v agar, using a spiral plater (Eddy jet spiral plater, IUL, Barcelona, Spain) with a sampling volume of 49.2 μ L. The BHI plates were then incubated for 24 h at 37°C. Subsequently, colony-forming units were counted. Results were logarithmically transformed and expressed as ln CFU/g. Each experiment was conducted independently in duplicates.

2.5. Modelling of microbial dynamics and parameter estimation

In this study, microbial dynamics of *S. Typhimurium* and *S. aureus* exhibited variable kinetics, e.g., growth, inactivation, as well as combined growth and inactivation. Therefore, each case was modelled with a different modelling approach.

When results exhibited **microbial growth**, the growth model of Baranyi and Roberts (1994) was fitted on the experimental data. This model is described by Equations 1, 2 and 3 (Baranyi and Roberts, 1994).

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

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

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

With $N(t)$ [CFU/g], the cell density at time t ; N_{max} [CFU/g], the maximum cell density; μ_{max} [1/h], the maximum specific growth rate; $Q(t)$ [-], the physiological state of the cells; $Q(0)$, a measure of the initial physiological state of the cells; and λ [h], the lag time of the cells.

In case results exhibited **microbial inactivation**, the inactivation model of Geeraerd et al. (2000) was fitted to the experimental data. This model is described by Equations 4, 5 and 6 (Geeraerd et al., 2000, 2005).

$$\frac{dN(t)}{dt} = - \left(\frac{1}{1 + C_c(t)} \right) \cdot k_{max} \cdot \left(1 - \frac{N_{res}}{N(t)} \right) \cdot N(t) \quad (4)$$

$$\frac{dC_c(t)}{dt} = -k_{max} \cdot C_c(t) \quad (5)$$

$$C_c(0) = e^{k_{max} S_L} - 1 \quad (6)$$

With $N(t)$ [CFU/g], the cell density at time t ; N_{res} [CFU/g], the residual cell density; k_{max} [1/h], the maximum specific inactivation rate; $C_c(t)$ [-], the concentration of a critical component (intracellular or extracellular) for cell survival; $C_c(0)$ [-], the initial concentration of this critical component; and S_L [h], the shoulder length representing the period the cells need to adapt to the stress. In cases where no shoulder S_L nor tail N_{res} appeared in the experimental data, these two parameters were set to zero value resulting to a log-linear expression for microbial inactivation. In this case only the N_0 and k_{max} parameters were estimated.

When experimental data demonstrated the occurrence of **both growth and inactivation** dynamics in the same set of experiments, the experimental data were fitted by combining the Baranyi and Roberts (1994) and the Geeraerd et al. (2000) models. In total eight parameters were estimated simultaneously: i) N_0 (the initial cell density at time $t=0$), (ii) N_{max} , (iii) μ_{max} , (iv) λ , (v) (t_{switch}), (vi) S_L , (vii) k_{max} and (viii) N_{res} . The time instance where N_{max} (growth model) was equal to the N_0 (inactivation model) was defined as the time of switch (t_{switch}). A graphical representation of the estimated parameters is provided in Figure 1.

Model parameters were estimated via the minimisation of the sum of squared errors, using the lsqnonlin routine of the Optimisation Toolbox of MatLab® version R2010b (The Mathworks, Inc., Natick, USA). For microbial growth, the initial cell population (N_0), the maximum specific growth rate (μ_{max}), the maximum cell population (N_{max}), and the lag time (λ) were estimated. For microbial inactivation, the initial cell population ($N_0 = N$ at t_{switch}), the maximum specific inactivation rate (k_{max}), the shoulder length (S_L), and the residual cell population (N_{res}) were estimated. The standard deviation (σ) of the estimated parameters was obtained from the square root of the main diagonal of the parameter covariance matrix $\mathbf{P}$. This matrix was calculated as $\{\mathbf{J}^T \cdot \mathbf{J}\} \cdot \text{MSE}$ with $\mathbf{J}$ the Jacobian matrix determined simultaneously, and MSE the mean sum of squared errors (Van Derlinden et al., 2008). The 95% Confidence Intervals (CI) of the estimated μ_{max} were also estimated for *S. Typhimurium* and *S. aureus* at 12°C, in order to compare with the predictions of ComBase.

The predictions of μ_{max} for *S. Typhimurium* and *S. aureus* were obtained by the ComBase (<http://www.combase.cc/>), web-based open access database for microbial behaviour. Specifically, the predictions of μ_{max} were obtained by using the ComBase Predictor for growth of *Salmonella* spp and *Staphylococcus aureus* at temperatures 4, 8 and 12°C and pH and a_w values as found for each of the model systems and presented in Table 1. The predictions of ComBase Predictor are based on experiments in liquid microbiological media and are

expressed in $\log(\text{conc})/\text{h}$ units. The ComBase predictions have been transformed to $\ln(\text{conc})/\text{h}$ units.

2.6. 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).

3. Results and Discussion

In Table 1 the physicochemical characteristics, e.g., pH and water activity (a_w) of the different model systems are demonstrated. The microbial dynamics of *S. Typhimurium* and *S. aureus* are represented in Figures 2 and 3 and the estimated growth parameters are provided in Tables 2 and 3, respectively.

3.1. *Salmonella Typhimurium* dynamics in/on variable (micro)structures

3.1.1. Storage temperature: 4°C

S. Typhimurium exhibited variable behaviour in/on the model systems depending on the incubation temperature. At 4°C, *S. Typhimurium* exhibited a log-linear inactivation at the three (micro)structures, liquids, emulsions and gelled emulsions. On aqueous gel, *S. Typhimurium* demonstrated an extended shoulder phase, prior to inactivation, as well as a tail phase, with a residual population after the inactivation phase. Nevertheless, it is worth to mention that the k_{max} on aqueous gels was the highest among the model systems. This extended lag and the fast

inactivation rate can be attributed to the differences among the model systems and mainly due to (micro)structural differences. Therefore, aqueous gel appears to be the most favourable environment for *S. Typhimurium* to survive at 4°C for longer period prior to inactivation. Among the (micro)structures where log-linear inactivation of *S. Typhimurium* occurred, the lowest k_{max} is observed in emulsion systems, then in liquids and finally on gelled emulsions. This observation implies that the highest stress is attributed to gelled emulsion model systems. This can be due to the lowest levels of a_w of these model systems and the presence of fat, which are both inhibitory factors. It has been reported that microbial cells grow in the water phase, but not in the fat phase (Wilson et al., 2002). Therefore, the presence of fat diminishes the contact surface of microbial cells with the nutritious phase. Between the emulsions and liquids, where planktonic growth occurs, liquids are more effective (micro)structures for the inactivation of *S. Typhimurium*. Fat presence has a cryoprotectant influence on the physiology of cells at 4°C (Knechtges, 2011; Saucier and Champagne, 2006), instead of just limiting the contact surface between microbial cells and the nutritious water phase.

3.1.2. Storage temperature 8°C

At 8°C, *S. Typhimurium* grew almost 1.5 ln on aqueous gel, gelled emulsion and emulsion systems and then exhibited inactivation. In liquid systems, a typical inactivation curve with shoulder and tail phases was observed. This observation implies that liquid systems at 8°C are the safest (micro)structures, causing inactivation of *S. Typhimurium*, without allowing regrowth. On the basis of ComBase predictions, 7°C was the minimum temperature for growth of *S. Typhimurium* in liquid systems. However, the experimental data of this study indicated that *S. Typhimurium* exhibits inactivation rather than growth even at 8°C in liquid systems. Although, ComBase predictions do not include the effect of composition, it is noteworthy that the liquids developed in this study have very low preservatives concentration to inhibit growth. Therefore, ComBase predictions might not be always accurate and require further evaluation.

Among the cases where outgrowth initially occurred, *S. Typhimurium* had an approximately 1.5 ln initial increase of its population on aqueous gels, while total inactivation followed at a high rate (k_{max}). The μ_{max} estimate of *S. Typhimurium* in this case was high, and this could be an artefact due to the very short exponential phase of growth with very few data points. In emulsion systems, a very similar behaviour of *S. Typhimurium* was observed, but a tail of residual population remained during storage time. On gelled emulsions, the population of *S. Typhimurium* increased by approximately 1.5 ln and then decreased approximately 1 ln reaching a residual population almost equal to the inoculum level. The behaviour of *S. Typhimurium*, when outgrowth and subsequent inactivation occurred, was modelled by combining the growth model of Baranyi and Roberts (Baranyi and Roberts, 1994) and the inactivation model of Geeraerd et al. (Geeraerd et al., 2000), both described in the materials and methods section of this paper. It is noteworthy to mention that microorganisms, typically after reaching a significant level of N_{max} , start dying (Rolfe et al., 2012). The death phase of the typical microbial growth curve is not modelled, mainly because of the lack of importance to food safety issues. However, in this study cells start inactivating before reaching significantly high maximum population. This observation is of major importance and worth to be modelled for aspects related to food safety. Finally, the gelled emulsion type of (micro)structure, at 8°C appeared to allow survival of *S. Typhimurium*. This observation highlighted the importance of considering all food intrinsic, as well as extrinsic factors, affecting microbial growth and/or inactivation in predictive models, applicable to microbiological food safety.

3.1.3. Storage temperature: 12°C

At 12°C, *S. Typhimurium* grew at a fast rate in/on all model systems. It is noteworthy that the differences in *S. Typhimurium* dynamics among the model systems became smaller than at lower temperatures. The estimated parameters are provided in Table 2. *S. Typhimurium* exhibited the highest μ_{max} in emulsions and liquids and finally on gelled emulsions and aqueous

gels. The model systems in which the fastest growth was observed were those where planktonic growth occurred (liquids and emulsions). However, the slowest growth was observed on aqueous gels and gelled emulsions, where surface growth occurred, in accordance to what has been found in relevant literature (Koutsoumanis et al., 2004). Moreover, the highest N_{max} of *S. Typhimurium* was reached on aqueous gels and gelled emulsions and then in liquids and emulsions. Similar sequence was observed for the N_{max} of *L. monocytogenes* in/on model systems of similar (micro)structures, but with different composition (Baka et al., 2016). Thereto, the model systems were vacuum packed and stored at 12°C, and their composition simulated Frankfurter sausages. This observation indicates that facultative anaerobic bacteria, e.g., *S. Typhimurium* and *L. monocytogenes*, reach higher maximum population when growing as surface colonies than planktonic cells, no matter the presence or absence of oxygen on their environment. Lag phase occurred only in emulsions, in/on all the other model systems the lag phase was equal to zero, as can be observed in Table 2. In this table, lag phase estimates, which approached the biologically lower bound of zero, appeared with unrealistically high standard error, as it was previously observed (Baka et al., 2014).

3.2. *Staphylococcus aureus* dynamics in/on variable (micro)structures

3.2.1. Storage temperature: 4 and 8°C

Both at 4°C and 8°C, *S. aureus* exhibited no growth in/on all different model systems. Specifically, a slight inactivation is observed in most cases at a level of 0.5 log, except aqueous gels and gelled emulsions at 8°C, where k_{max} is zero, as can be seen also in Table 3. The fastest inactivation of *S. aureus* occurred in liquids, while emulsions, gelled emulsions and aqueous gels had similar k_{max} values, as presented in Figure 3 and Table 3. In fact the modelled inactivation could also be considered as survival of *S. aureus* throughout the storage period in/on all model systems.

3.2.2. Storage temperature: 12°C

At 12°C, *S. aureus* grew throughout the storage period both on aqueous gels and in liquid systems. The fastest growth occurred on aqueous gels and in liquids, in which *S. aureus* had similar μ_{max} estimates. In emulsion systems *S. aureus* exhibited an extended lag phase (Table 3), but then it started growing at a μ_{max} similar to liquids and aqueous gels. On gelled emulsions, *S. aureus* cells grew with the lowest μ_{max} and reached the lowest N_{max} . At this storage temperature, where growth occurred for *S. aureus*, the most favourable environment for growth was created by the model systems without fat and with the highest a_w values, e.g., liquids and aqueous gels. This storage temperature is still suboptimal for growth for *S. aureus*, but not very close to the minimum of growth which is 7.5°C. Therefore, fat presence acted mostly as disturbing factor for growth rather than protective as in the previous storage temperatures.

3.3. Comparison between *Salmonella* Typhimurium and *Staphylococcus aureus* dynamics

Overall, *S. Typhimurium* appeared to be less resistant at 4°C than *S. aureus* by comparing their k_{max} values (Tables 2 and 3). At 8°C, the comparison between the two species is not straightforward, as *S. Typhimurium* first grows and then inactivates, while *S. aureus* only inactivates. For *S. Typhimurium* the sequence of favourable conditions created by the model systems for growth and/or survival was dependent on the storage temperature, for temperatures between 4 and 12°C. Yet, for *S. aureus*, it was observed that this temperature dependence occurred between a tighter region of 8 and 12°C. Overall, *S. aureus* was favoured by surface inoculation, while *S. Typhimurium* did not exhibit conclusive dynamics. In a study where the dynamics of *Listeria monocytogenes* were studied in/on model systems of variable (micro)structures simulating the composition of Frankfurter sausages (Baka et al., 2016), *L. monocytogenes* exhibited growth at all temperatures and a complicated influence of storage temperature was also involved altering the sequence of preferential growth in/on the model systems. Nevertheless, in the latter study, the addition of fat limited the μ_{max} of *L.*

monocytogenes to lower values at all storage temperatures (4, 8 and 12°C). In this study, for *S. Typhimurium* and *S. aureus* the influence of the fat addition is not so conclusive.

3.4. ComBase comparison dynamics

The maximum specific growth rate of *S. Typhimurium* and *S. aureus*, as predicted by ComBase for the specific pH, a_w and storage temperature of each model system, are provided in Table 4. Additionally, the 95% confidence intervals (CI) of the estimated μ_{max} by the Baranyi and Roberts model at 12°C for both species are provided. The 95% CI for the estimated μ_{max} at 8°C are not provided, as inactivation or, growth and inactivation occurred. Therefore, no μ_{max} was estimated at these conditions. In Table 4, the predictions at 4°C are not provided as the minimum temperature for growth on the basis of ComBase was 7 and 7.5°C for *S. Typhimurium* and *S. aureus*, respectively. The comparison between the predictions of ComBase and the estimated parameters, as well as their 95% CI, of the experimental data of this study indicate that ComBase predictions overestimate the growth dynamics of both species, when cultured at the same pH and a_w values. Since the predictions of ComBase are based on liquid microbiological media, while the estimated parameters of this study are based on model systems of variable (micro)structures and subsequently to compositional variations, the differences in dynamics are a consequence of the previously mentioned variations. The influence of compositional variation is mainly profound when comparing ComBase predictions (which are obtained by experiments in liquid microbiological media) with the estimated parameters of this study in the liquid model systems. Between the compared values, the (micro)structural effect is neglected (planktonic growth in homogeneous liquid systems in both cases), therefore the differences in microbial dynamics can be considered a results of compositional variations. However, when comparing the dynamics in/on the other three model systems with the ComBase predictions, the interpretation of results becomes more complicated as both (micro)structural and compositional variations are involved. It is worth to mention that a_w values used in ComBase

are defined mainly by NaCl concentrations and not by proteins or fat, which influence the a_w of the model systems in this study. In the same table, the profound influence of a_w can also be observed. The differences in μ_{max} of ComBase predictions are mainly caused due to the differences in a_w . Liquids and aqueous gels have very similar a_w values (0.9852 and 0.9853, respectively), as well as emulsions and gelled emulsion systems (0.9774 and 0.9777, respectively). Likewise, the μ_{max} values of the liquids are very similar to aqueous gels and the μ_{max} of emulsions are very similar to the gelled emulsions. pH is very similar among the model systems, so it does not appear to have any influence. The influence of the a_w variation among the model systems is also reflected on their estimated dynamics from the experimental data. Most similarities in dynamics are observed between (i) liquids and aqueous gels and (ii) emulsions and gelled emulsions.

4. Conclusion

In this study, the complexity of the influence of food (micro)structure on the growth/inactivation dynamics of pathogenic species, such as *S. Typhimurium* and *S. aureus* was illustrated. The influence of (micro)structure was studied by means of model systems, approaching in composition fish patés. The influence of (micro)structure on microbial dynamics was confirmed by the comparison of μ_{max} values estimated in the current study compared with the predictions of ComBase Predictor. Overall, ComBase overestimated microbial growth at the same pH, a_w and storage temperature. When the storage temperature changes, the influence of the food (micro)structure on both microorganisms dynamics alters as well. Such observation suggests a further investigation of the influence of storage temperature, at a more wide range, in combination with food (micro)structure and other factors, e.g., physicochemical and compositional properties, which are consequential from the (micro)structural variability.

ACKNOWLEDGEMENTS

This work was supported by project PFV/10/002 (Center of Excellence OPTEC-Optimization in Engineering) of the KU Leuven Research Fund, project G.0930.13 and KAN.15189.13N of the Fund for Scientific Research-Flanders, and the Belgian Program on Interuniversity Poles of Attraction, initiated by the Belgian Federal Science Policy Office (IAP Phase VII - 19 DYSCO).

References

- Aquerreta, Y., Astiasarán, I., Mohino, A., Bello, J., 2002. Composition of pâtés elaborated with mackerel flesh (*Scomber scombrus*) and tuna liver (*Thunnus thynnus*): Comparison with commercial fish pâtés. *Food Chem.* 77, 147–153. doi:10.1016/S0308-8146(01)00310-7
- Baka, M., Noriega, E., Mertens, L., Van Derlinden, E., Van Impe, J.F.M., 2014. Protective role of indigenous *Leuconostoc carnosum* against *Listeria monocytogenes* on vacuum packed Frankfurter sausages at suboptimal temperatures. *Food Res. Int.* 66, 197–206. doi:10.1016/j.foodres.2014.08.011
- Baka, M., Noriega, E., Van Langendonck, K., Van Impe, J.F., 2016. Influence of food intrinsic complexity on *Listeria monocytogenes* growth in/on vacuum-packed model systems at suboptimal temperatures. *Int. J. Food Microbiol.* 235, 17–27. doi:10.1016/j.ijfoodmicro.2016.06.029
- Baranyi, J., Roberts, T.A., 1994. A dynamic approach to predicting bacterial growth in food. *Int. J. Food Microbiol.* 23, 277–294. doi:10.1016/0168-1605(94)90157-0
- Cooke, F., Threlfall, E., Wain, J., 2007. *Salmonella: Molecular Biology and Pathogenesis*, in: Rhen, M., Maskell, D., Mastroeni, P., Threlfall, J. (Eds.), *Salmonella Molecular Biology and Pathogenesis*. Horizon Scientific Press, Wymondham, Norfolk (UK), pp. 1–29.

- Echarte, M., Conchillo, A., Ansorena, D., Astiasarán, I., 2004. Evaluation of the nutritional aspects and cholesterol oxidation products of pork liver and fish patés. *Food Chem.* 86, 47–53. doi:10.1016/j.foodchem.2003.08.027
- EFSA and ECDC, 2015. Trends and sources of zoonoses , zoonotic agents and food-borne. *EFSA J.* 13, 3991, 162pp. doi:10.2903/j.efsa.2015.3991
- EUMOFA, 2015. The EU fish market [WWW Document]. URL http://ec.europa.eu/newsroom/mare/itemdetail.cfm?item_id=27723 (accessed 2.16.16).
- Geeraerd, a. H., Herremans, C.H., Van Impe, J.F., 2000. Structural model requirements to describe microbial inactivation during a mild heat treatment. *Int. J. Food Microbiol.* 59, 185–209. doi:10.1016/S0168-1605(00)00362-7
- Geeraerd, A.H., Valdramidis, V.P., Van Impe, J.F., 2005. GInaFiT, a freeware tool to assess non-log-linear microbial survivor curves. *Int. J. Food Microbiol.* 102, 95–105. doi:10.1016/j.ijfoodmicro.2004.11.038
- Gonçalves, A.A., Ribeiro Duarte, J.L., 2008. Do phosphates improve the seafood quality? Reality and legislation. *Panam. J. Aquat. Sci.* 3, 237–247.
- Greig, J.D., Ravel, A., 2009. Analysis of foodborne outbreak data reported internationally for source attribution. *Int. J. Food Microbiol.* 130, 77–87. doi:10.1016/j.ijfoodmicro.2008.12.031
- Knechtges, P.L., 2011. *Food Safety: Theory and Practice*. Jones & Bartlett Publishers, Burlington, MA.
- Koutsoumanis, K.P., Kendall, P.A., Sofos, J.N., 2004. A comparative study on growth limits of *Listeria monocytogenes* as affected by temperature, pH and aw when grown in suspension or on a solid surface. *Food Microbiol.* 21, 415–422.

doi:10.1016/j.fm.2003.11.003

McDonald, K., Sun, D.W., 1999. Predictive food microbiology for the meat industry: a review.

Int. J. Food Microbiol. 52, 1–27. doi:10.1016/S0168-1605(99)00126-9

Mertens, L., Van Derlinden, E., Dang, T.D.T., Cappuyns, A.M., Vermeulen, A., Debevere, J.,

Moldenaers, P., Devlieghere, F., Geeraerd, A.H., 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 Microbiol. 28, 736–45.

doi:10.1016/j.fm.2010.05.032

Mohamed Hatha, A., 1997. Prevalence of *Salmonella* in fish and crustaceans from markets in

Coimbatore, South India. Food Microbiol. 14, 111–116. doi:10.1006/fmic.1996.0070

Rahimi, E., Shakerian, A., Falavarjani, A.G., 2013. Prevalence and antimicrobial resistance of

Salmonella isolated from fish, shrimp, lobster, and crab in Iran. Comp. Clin. Path. 22, 59–62. doi:10.1007/s00580-011-1368-3

Rolfe, M.D., Rice, C.J., Lucchini, S., Pin, C., Thompson, A., Cameron, A.D.S., Alston, M.,

Stringer, M.F., Betts, R.P., Baranyi, J., Peck, M.W., Hinton, J.C.D., 2012. Lag phase is a distinct growth phase that prepares bacteria for exponential growth and involves transient metal accumulation. J. Bacteriol. 194, 686–701. doi:10.1128/JB.06112-11

Ross, T., McMeekin, T. a, 1994. Predictive microbiology. Int. J. Food Microbiol. 23, 241–264.

doi:10.1016/0168-1605(94)90155-4

Saito, E., Yoshida, N., Kawano, J., Shimizu, A., Igimi, S., 2011. Isolation of *Staphylococcus*

aureus from raw fish in relation to culture methods. J. Vet. Med. Sci. 73, 287–292.

doi:10.1292/jvms.10-0198

Saucier, L., Champagne, C.P., 2006. Applications of Cell Immobilisation Biotechnology.

Springer Science & Business Media, Dordrecht, The Netherlands.

- Simopoulos, A., 1996. Handbook of lipids in human nutrition, in: Gene, A. (Ed.), Essentials of Food Science. CRC Press, Boca Raton, Florida, pp. 51–73.
- Sutton Scott, 2011. Accuracy of plate counts. J. Valid. Technol. 17, 42–46.
- Tahergorabi, R., Beamer, S.K., Matak, K.E., Jaczynski, J., 2012. Functional food products made from fish protein isolate recovered with isoelectric solubilization/precipitation. LWT - Food Sci. Technol. 48, 89–95. doi:10.1016/j.lwt.2012.02.018
- Vaclavik, V., Christian, E., 2014. Essentials of Food Science, in: Vaclavik, V., Elizabeth W, C. (Eds.), . Springer Science & Business Media, New York (US), pp. 133–172.
- Van Derlinden, E., Bernaerts, K., Van Impe, J.F., 2008. Accurate estimation of cardinal growth temperatures of Escherichia coli from optimal dynamic experiments. Int. J. Food Microbiol. 128, 89–100. doi:10.1016/j.ijfoodmicro.2008.07.007
- Van Impe, J., Poschet, F., Geeraerd, A., Vereecken, K., 2005. Towards a novel class of predictive microbial growth models. Int. J. Food Microbiol. 100, 97–105. doi:10.1016/j.ijfoodmicro.2004.10.007
- Wilson, P.D., Brocklehurst, T., Arino, S., Thuault, D., Jakobsen, M., Lange, M., Farkas, J., Wimpenny, J.W., Van Impe, J., 2002. Modelling microbial growth in structured foods: towards a unified approach. Int. J. Food Microbiol. 73, 275–289. doi:10.1016/S0168-1605(01)00660-2

Figure captions

Figure 1. Graphical representation of the estimated parameters N_0 : Initial inoculum level, μ_{max} : the maximum specific growth rate, λ : the lag phase, $N(t=t_{switch})$: the cell population at the time of switch, S_L : the shoulder length, k_{max} : the maximum specific inactivation rate, N_{res} : the residual population, t_{switch} : the time of switch when both microbial growth and inactivation occurred.

Figure 2. Growth and/or inactivation dynamics of *Salmonella* Typhimurium in/on liquids, emulsions, aqueous gels, and gelled emulsions 4 (a), 8 (b) and 12°C (c). The average experimental data conducted in duplicates and their standard deviation (error bars) in/on: the liquids are represented with (*), emulsions with (●), aqueous gels with () and gelled emulsions with (+). The Geeraerd et al. (complete or log-linear version), the Baranyi and Roberts and a combination of both models are fitted on the experimental data according to the experimental data.

Figure 3. Growth and/or inactivation dynamics of *Staphylococcus aureus* in/on liquids, emulsions, aqueous gels, and gelled emulsions 4 (a), 8 (b) and 12°C (c). The average experimental data conducted in duplicates and their standard deviation (error bars) in/on: the liquids are represented with (*), emulsions with (●), aqueous gels with () and gelled emulsions with (+). The Geeraerd et al. (log-linear version) and the Baranyi and Roberts models are fitted on the experimental data according to the experimental data.

Highlights

- *Staphylococcus aureus* is favoured by colonial morphology on solid surfaces
- Among model systems, *S. Typhimurium* dynamics are influenced by T
- Interaction of food intrinsic & extrinsic factors was found on microbial dynamics

Figure 1

Figure 2

Figure 3

Table 1. Composition in (%w/w) and physicochemical characteristics of model systems

Ingredients and physicochemical characteristics	Description of Ingredients	Liquid	Emulsion	Aqueous gel	Gelled emulsion
Water	Distilled water	88.78	60.43	87.58	59.23
Protein	Fish protein, ProGo™, Hofseth Biocare ASA, Ålesund, Norway	7.66	7.66	7.66	7.66
Fat	Animal fat (local butcher)	0.00	26.85	0.00	26.85
Starch	Soluble Powder, Fisher Scientific, UK	2.00	2.00	2.00	2.00
Agar	Agar technical (No.3), Oxoid, UK	0.00	0.00	0.70	0.70
Guar	Sigma Aldrich, Germany	0.00	0.00	0.50	0.50
Emulsifier	Citrem 3302, Paalgaard, Denmark	0.00	1.50	0.00	1.50
Sodium chloride	Sigma Aldrich, Germany	1.06	1.06	1.06	1.06
Pentasodium triphosphate	Sigma Aldrich, Germany	0.50	0.50	0.50	0.50
pH	-	6.34	6.23	6.32	6.17
a_w	-	0.9852	0.9774	0.9853	0.9777

Table 2. Estimated Parameters (EP) for growth and/or inactivation and their Standard Deviation (SD) for *Salmonella* Typhimurium at three different storage temperatures in/on the studied model systems.

Model system	Parameter	Unit	EP $\pm$ SD (4°C)	EP $\pm$ SD (8°C)	EP $\pm$ SD (12°C)
Liquid	N _o	log(CFU/g) or ln(CFU/g) **	2.0501 $\pm$ 0.0488 ^a	1.968 $\pm$ 0.0461	4.500 $\pm$ 0.481 ^a
	N _{max}	ln(CFU/g)	-	-	19.769 $\pm$ 0.284 ^a
	μ _{max}	h ⁻¹	-	-	0.057 $\pm$ 0.003 ^a
	λ	h	-	-	0.000 $\pm$ 11.836 ^a
	S _L	h	-	271.93 $\pm$ 17.47	-
	k _{max}	h ⁻¹	0.0034 $\pm$ 0.0002 ^a	0.038 $\pm$ 0.013	-
	N _{res}	log(CFU/g)	-	0.788 $\pm$ 0.088	-
	MSE*	-	0.0132	0.0139	0.3350
Emulsion	N _o	log(CFU/g) or ln(CFU/g) **	1.8109 $\pm$ 0.0547 ^b	4.159 $\pm$ 0.155 ^a	3.424 $\pm$ 0.483 ^b
	N _{max}	ln(CFU/g)	-	5.914 $\pm$ 0.179 ^{ab}	19.067 $\pm$ 0.251 ^b
	μ _{max}	h ⁻¹	-	0.080 $\pm$ 0.033 ^a	0.059 $\pm$ 0.002 ^a
	λ	h	-	44.82 $\pm$ 8.05 ^{ab}	12.710 $\pm$ 1.449 ^a
	t _{switch}	h	-	116.13 $\pm$ 15.25 ^a	-
	S _L	h	-	32.90 $\pm$ 81.37 ^a	-
	k _{max}	h ⁻¹	0.0017 $\pm$ 0.0002 ^a	0.026 $\pm$ 0.012 ^a	-
	N _{res}	log(CFU/g)	-	1.242 $\pm$ 0.141 ^a	-
Aqueous gel	MSE*	-	0.0145	0.0277	0.2539
	N _o	log(CFU/g) or ln(CFU/g) **	2.3500 $\pm$ 0.0324 ^c	4.876 $\pm$ 0.284 ^b	4.701 $\pm$ 0.463 ^a
	N _{max}	ln(CFU/g)	-	6.337 $\pm$ 0.127 ^a	21.997 $\pm$ 0.336 ^c
	μ _{max}	h ⁻¹	-	0.168 $\pm$ 0.484 ^a	0.045 $\pm$ 0.002 ^b
	λ	h	-	23.665 $\pm$ 18.480 ^a	0.000 $\pm$ 14.442 ^a
	t _{switch}	h	-	124.75 $\pm$ 0.28 ^a	-
	S _L	h	527.633 $\pm$ 22.939	1.84 $\pm$ 5.17 ^a	-
	k _{max}	h ⁻¹	0.0121 $\pm$ 0.0014 ^b	0.029 $\pm$ 0.016 ^a	-
Gelled emulsion	N _{res}	log(CFU/g)	0.6189 $\pm$ 0.0809	0.000 $\pm$ 2.305 ^a	-
	MSE*	-	0.0057	0.0804	0.3525
	N _o	log(CFU/g) or ln(CFU/g) **	2.052 $\pm$ 0.073 ^a	3.967 $\pm$ 0.111 ^a	3.302 $\pm$ 0.430 ^b
	N _{max}	ln(CFU/g)	-	5.495 $\pm$ 0.288 ^b	21.145 $\pm$ 0.280 ^c
	μ _{max}	h ⁻¹	-	0.037 $\pm$ 0.016 ^a	0.050 $\pm$ 0.002 ^b
	λ	h	-	59.789 $\pm$ 13.139 ^b	0.000 $\pm$ 11.978 ^a
	t _{switch}	h	-	171.75 $\pm$ 0.00 ^b	-
	S _L	h	-	100.52 $\pm$ 304.32 ^a	-
	k _{max}	h ⁻¹	0.0054 $\pm$ 0.0004 ^c	0.052 $\pm$ 0.031 ^a	-
	N _{res}	log(CFU/g)	-	2.023 $\pm$ 0.057 ^a	-
	MSE*	-	0.0209	0.0147	0.2896

*MSE: Mean Square Error indicate the goodness of fit of the model on the data.

**log(CFU/g) refers to inactivation data, while ln(CFU/g) to data where either only growth or both growth and inactivation occurred.

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

Journal homepage: <https://www.sciencedirect.com/science/journal/S01681605/240>

Original file available at: <https://www.sciencedirect.com/science/article/pii/S016816051630397X>

Table 3. Estimated Parameters (EP) for growth and/or inactivation and their Standard Deviation (SD) for *Staphylococcus aureus* at three different storage temperatures in/on the studied model systems.

Model system	Parameter	Unit	EP $\pm$ SD (4°C)	EP $\pm$ SD (8°C)	EP $\pm$ SD (12°C)
Liquid	N_0	log(CFU/g) or ln(CFU/g)**	2.1436 $\pm$ 0.1044 ^{ab}	2.0894 $\pm$ 0.0278 ^a	4.7765 $\pm$ 0.1466 ^a
	N_{max}	ln(CFU/g)	-	-	17.1601 $\pm$ 0.1818 ^a
	μ_{max}	h ⁻¹	-	-	0.0378 $\pm$ 0.0015 ^a
	λ	h	-	-	72.215 $\pm$ 9.613 ^a
	k_{max}	h ⁻¹	0.0039 $\pm$ 0.0009 ^a	0.0016 $\pm$ 0.0002 ^a	-
	MSE*	-	0.0317	0.0031	0.1080
Emulsion	N_0	log(CFU/g) or ln(CFU/g)**	1.9929 $\pm$ 0.0351 ^a	2.0884 $\pm$ 0.0508 ^a	4.0320 $\pm$ 0.1240 ^b
	N_{max}	ln(CFU/g)	-	-	23.00 $\pm$ 0.00 ^b
	μ_{max}	h ⁻¹	-	-	0.0367 $\pm$ 0.0042 ^a
	λ	h	-	-	385.4 $\pm$ 14.0 ^b
	k_{max}	h ⁻¹	0.0016 $\pm$ 0.0003 ^b	0.0012 $\pm$ 0.0005 ^a	-
	MSE*	-	0.0036	0.0095	0.1311
Aqueous gel	N_0	log(CFU/g) or ln(CFU/g)**	2.2564 $\pm$ 0.0460 ^b	2.2929 $\pm$ 0.00667 ^b	4.3519 $\pm$ 0.2986 ^{ab}
	N_{max}	ln(CFU/g)	-	-	18.761 $\pm$ 0.186 ^c
	μ_{max}	h ⁻¹	-	-	0.0346 $\pm$ 0.0012 ^a
	λ	h	-	-	5.5785 $\pm$ 13.5496 ^a
	k_{max}	h ⁻¹	0.0009 $\pm$ 0.0004 ^b	0.0000 $\pm$ 0.0005 ^b	-
	MSE*	-	0.0065	0.0178	0.1684
Gelled emulsion	N_0	log(CFU/g) or ln(CFU/g)**	2.0334 $\pm$ 0.0615 ^a	2.147 $\pm$ 0.0597 ^{ab}	4.4914 $\pm$ 0.3168 ^{ab}
	N_{max}	ln(CFU/g)	-	-	9.1134 $\pm$ 0.2415 ^d
	μ_{max}	h ⁻¹	-	-	0.0148 $\pm$ 0.0041 ^b
	λ	h	-	-	0.0000 $\pm$ 66.4102 ^a
	k_{max}	h ⁻¹	0.0009 $\pm$ 0.0005 ^b	0.0000 $\pm$ 0.0005 ^b	-
	MSE*	-	0.0115	0.0143	0.2326

*MSE: Mean Square Error indicates the goodness of fit of the model

**log(CFU/g) refers to 4 and 8°C (inactivation data), while ln(CFU/g) to 12°C (growth data)

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

Table 4. μ_{max} predictions of ComBase on the basis of pH, a_w and storage temperature of the different model systems and 95% confidence intervals (CI) of the estimated μ_{max} at 12 °C for *Salmonella* Typhimurium and *Staphylococcus aureus*.

Microorganism	<i>Salmonella</i> Typhimurium		<i>Staphylococcus aureus</i>	
Model system	$\mu_{\max}$ (1/h) at 8 (°C)			
Liquid	0.0368		0.0322	
Emulsion	0.0276		0.0299	
Aqueous gel	0.0368		0.0322	
Gelled emulsion	0.0276		0.0299	
	$\mu_{\max}$ (1/h) at 12 (°C)	95% CI of estimated $\mu_{\max}$ at 12 (°C)	$\mu_{\max}$ (1/h) at 12 (°C)	95% CI of estimated $\mu_{\max}$ at 12 (°C)
Liquid	0.0967	[0.0517, 0.0626]	0.0944	[0.0346, 0.0410]
Emulsion	0.0714	[0.0537, 0.0646]	0.0852	[0.0272, 0.0461]
Aqueous gel	0.0967	[0.0418, 0.0492]	0.0944	[0.0320, 0.0372]
Gelled emulsion	0.0736	[0.0464, 0.0535]	0.0875	[0.0060, 0.0235]