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Impact of food model (micro)structure on the microbial inactivation efficacy of cold atmospheric plasma

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ABSTRACT

The large potential of cold atmospheric plasma (CAP) for food decontamination has recently been recognized. Room-temperature gas plasmas can decontaminate foods without causing undesired changes. This innovative technology is a promising alternative for treating fresh produce. However, more fundamental studies are needed before its application in the food industry. The impact of the food structure on CAP decontamination efficacy of *Salmonella* Typhimurium and *Listeria monocytogenes* was studied. Cells were grown planktonically or as surface colonies in/on model systems. Both microorganisms were grown in lab culture media in petri dishes at 20°C until cells reached the stationary phase. Before CAP treatment, cells were deposited in a liquid carrier, on a solid(like) surface or on a filter. A dielectric barrier discharge reactor generated helium-oxygen plasma, which was used to treat samples up to ten minutes. Although *L. monocytogenes* is more resistant to CAP treatment, similar trends in inactivation behavior as for *S.* Typhimurium are observed, with log reductions in the range [1.0-2.9] for *S.* Typhimurium and [0.2-2.2] for *L. monocytogenes*. For both microorganisms, cells grown planktonically are easily inactivated, as compared to surface colonies. More stressing growth conditions, due to cell immobilization, result in more resistant cells during CAP treatment. The main difference between the inactivation support systems is the absence or presence of a shoulder phase. For experiments in the liquid carrier, which exhibit a long shoulder, the plasma components need to diffuse and penetrate through the medium. This explains the higher efficacies of CAP treatment on cells deposited on a solid(like) surface or on a filter. This research demonstrates that the food structure influences the cell inactivation behavior and efficacy of CAP, and indicates that food intrinsic factors need to be accounted when designing plasma treatment.

Keywords: cold atmospheric gas plasma; food model (micro)structure; growth morphology; inactivation support system; sublethal injury

1. INTRODUCTION

Over the last century, the government and the food industry have invested a significant amount of money and effort in food safety and quality research (e.g., Better Training for Safer Food, CHAFEA or From Farm to Fork, DG Health and Consumers, both European Commission initiatives). However, more than 300,000 food poisonings have been reported in 2013 in the EU. *Salmonella* and *Campylobacter* have been the most often reported foodborne pathogens. As in previous years, the most severe fatality rate (15.6%) among the cases was found for *Listeria monocytogenes* (EFSA and ECDC, 2015). To prevent the occurrence of pathogenic microorganisms in foods, thermal treatments, like pasteurization, are very effective for inactivating most microorganisms. However, thermal treatments influence the food quality with nutritional losses and changes in organoleptic properties as unwanted side effects (Paull et al., 2000). Additionally, consumer demand towards 'fresh-like' and natural foods, preferably with a long shelf-life and minimal preparation before consumption has increased. Fruits and vegetables, often consumed raw, have gained popularity and constitute a large part of our diet. To produce microbiologically safe and stable food products that meet the above requirements, a search for mild decontamination procedures has emerged. Traditional decontamination treatments for fruits and vegetables have focused on washing treatments combined with chemical biocides that are applied either pre-harvest, post-harvest or during processing (Aharoni et al., 1997; Goodburn and Wallace, 2013). Some adverse effects, such as the formation of carcinogenic halogenated by-products, e.g., chlorine, and the additional impetus of reducing water usage as well as chemical emissions have turned the focus of the food industry towards non-thermal physical decontamination technologies including cold atmospheric plasma (CAP) (Chen et al., 2010; Laroussi et al., 2000).

In general, CAP is generated by applying a high voltage to a gas stream. The gas molecules or atoms become ionized once a certain threshold is exceeded, resulting in mixtures of electrons,

ions, atomic species, free radicals and UV photons, all able to inactivate microorganisms (Deng et al., 2006; Millan-Sango et al., 2015; Perni et al., 2007a). Some of the most important process parameters are the plasma power, the voltage, the frequency and the gas flow. Regarding the gas composition, the presence of oxygen is proved to enhance the killing efficacy (Fernandez and Thompson, 2012; Perni et al., 2007a). Another important processing parameter concerns the reactor itself. Different plasma set ups exist, but two frequently used types are the plasma jet and the dielectric barrier discharge (DBD) electrode (Ehlbeck et al., 2011). While the plasma jet can be used to treat complex geometries, the DBD electrode is able to treat more extensive samples. For both set ups, the sample can be treated directly or indirectly. Direct treatment indicates that all plasma generated species, including the charged particles, come in contact with the sample. This is not valid for indirect treatment, with the sample placed at a distance from the plasma discharge (Fernandez and Thompson, 2012; Fridman et al., 2007; Olszewski et al., 2014). One of the possibilities is generating the plasma inside a closed reactor, which keeps the reactive plasma environment inside the system. Working inside a closed reactor prevents the possibility of recontamination and since the formed radicals remain inside the system, they remain active. Important advantages of using CAP for food treatment are (1) low temperature during treatment, (2) low energy requirement, (3) short treatment time and (4) no residues remaining on the surface of the treated product since chemical species formed during plasma treatment are highly reactive and very short-lived (Moisan et al., 2001). Over the last ten years, multiple research groups have studied the potential of CAP to be applied in the food industry. Limitations of previous studies are their focus on specific target microorganisms in relation to specific food products (Fernandez et al., 2013; Gurol et al., 2012; Kim et al., 2011; Selcuk et al., 2008). More general studies focusing on a more fundamental evaluation, e.g., the influence of food structural properties on the CAP inactivation efficacy, are mostly lacking.

In the present work, the impact of the food structure on the efficacy of CAP inactivation of *Salmonella* Typhimurium and *Listeria monocytogenes* was studied. The food (micro)structure can be defined as the organization of elements within a food and their interactions (Aguilera et al., 2000). During growth, the intrinsic food structure is reflected in the growth morphology, i.e., planktonic cells or surface colonies. Additionally, the influence of the support system, and thus the influence of the food structure during the inactivation was studied: cells were inactivated on a solid(like) surface or in a liquid carrier, mimicking the treatment of various food structures and investigating the possibilities of CAP to treat different types of food products.

2. MATERIALS AND METHODS

2.1 Experimental plan

The influence of the food structure on the CAP efficacy of *Salmonella* Typhimurium and *Listeria monocytogenes* was studied. During growth, cells were either grown planktonically or as surface colonies. Next to this, cells from the preculture were also treated. Surface colonies were promoted by the addition of 5% (w/v) gelatin to the growth medium. This amount of gelatin was selected based on knowledge from previous studies, and is often found in food (e.g., meat products) (Smet et al., 2015a, 2015b; Theys et al., 2008). Secondly, the influence of the support system during the inactivation itself was included. Cells were inactivated on a solid(like) surface or in a liquid carrier, mimicking treatment of solid and liquid food products. As a control, inactivation experiments on a filter surface were performed. To clarify all different combinations tested, reference is made to Figure 1. All experiments were conducted in duplicate.

2.2 Microorganisms and preculture conditions

Salmonella enterica serovar Typhimurium SL1344 was kindly provided by the Institute of Food Research (IFR, Norwich, UK). The culture was stored at -80°C in Tryptone Soya Broth (TSB (Oxoid LTd., Basingstoke, UK)) supplemented with 25% (v/v) glycerol (Acros Organics, NJ, USA). For every experiment, a fresh purity plate was prepared from the frozen stock culture by spreading a loopful onto a Tryptone Soya Agar plate (TSA (Oxoid Ltd., Basingstoke, UK)) incubated at 37°C for 24 h. One colony from this plate was transferred into 20 mL TSB and incubated under static conditions at 37°C for 8 h (Binder KB series incubator; Binder Inc., NY, USA). Next, 200 µL from this stationary phase culture was added to 20 mL of fresh TSB and incubated under the same conditions for 16 h.

Listeria monocytogenes LMG 13305 was obtained from the Belgian Co-ordinated Collections of Microorganisms (BCCM, Ghent, Belgium). The culture was stored at -80°C in TSB supplemented with 0.6% (w/v) yeast extract (Merck, Darmstadt, Germany) (TSBYE). For each experiment, a new purity plate was prepared on Brain Heart Infusion (BHI (Oxoid Ltd., Basingstoke, UK)) supplemented with 1.2% (w/v) agar (Agar technical n°3, Oxoid Ltd., Basingstoke, UK) and incubated for 24 h at 37°C. One colony from the purity plate was transferred into 20 mL BHI, incubated at 37°C for 8 h under static conditions, refreshed in BHI and incubated again for 16 h.

Cell cultivation under the above defined conditions yielded early-stationary phase populations for both *S.* Typhimurium and *L. monocytogenes*, at about 10^9 CFU/mL. These cultures were used to inoculate the corresponding media at the appropriate concentration.

2.3 Growth phase prior to CAP inactivation

2.3.1 Liquid systems: preparation and planktonic growth conditions

Planktonic cells were grown at 20°C, under optimal experimental conditions regarding pH and salt concentration (pH 7.4, 0% (w/v) NaCl), until the early stationary phase was reached. Results from preliminary growth experiments were used to verify this point on the growth curve. For *S.* Typhimurium, the growth medium consisted of TSB without dextrose (Becton, NJ, USA) of which the pH (DocuMeter, Sartorius, Goettingen, Germany) was 7.4. BHI was used for *L. monocytogenes*. Planktonic growth was performed in petri dishes (diameter 5.5 cm) filled with 7 mL of the growth medium inoculated at 10^3 CFU/mL. This cell density was obtained by serial decimal dilutions of stationary phase cells, using dilution medium with the same pH and salt concentration as the final growth conditions. After shaking, the inoculated growth medium was dispensed into petri dishes. For each experiment about 10 petri dishes were placed in a temperature controlled incubator (KB 8182, Termaks, Bergen, Norway) at 20°C under static conditions. Previous studies indicate that the rate of inactivation is independent of the growth temperature (Fernandez et al., 2013). Therefore, 20°C, mimicking the temperature during the CAP treatment, was selected. Cells were grown until the early stationary phase was reached, which was confirmed by cell counts. Early stationary phase cells were treated with CAP since it has been reported that these cells have the highest resistance and are most often encountered in a natural environment (Hurst, 1977; Rees et al., 1995).

2.3.2 Solid(like) systems: preparation and (surface) colonial growth conditions

Surface colonies were also grown at 20°C until the early stationary phase, under optimal pH and without the addition of salt. Gelatin at 5% (w/v) (gelatin from bovine skin, type B, Sigma-Aldrich, MO, USA) was added to TSB or BHI. After heating for 20 min at 60°C in a

thermostatic water bath (GR150-S12, Grant Instruments Ltd, Shepreth, UK), the gelatin melted and the medium was adapted by the addition of 5 M KOH (Acros Organics, NJ, USA) to pH 7.4. The medium was then filter-sterilized using a 0.2 mm filter (Filtertop, 150 mL filter volume, 0.22 µm, TPP, Switzerland), kept liquid at 60°C, and 7 mL was pipetted into sterile petri dishes (diameter 5.5 cm). After solidification, the plates were surface inoculated at approximately 3.0 x 10^2 CFU/cm^2 (surface area 23.8 cm^2, corresponding to 10^3 CFU/mL). This concentration was obtained by serial decimal dilution of stationary phase cells with the appropriate dilution medium, followed by spreading 20 µL of the corresponding dilution onto each petri dish. After being sealed, plates were placed simultaneously in a temperature controlled incubator at 20°C under static conditions and grown until the stationary phase was reached.

2.4 Sample inoculation for CAP inactivation

Once the cells, grown as either planktonic cells or surface colonies, reached the stationary growth phase, they were transferred to the appropriate inactivation support system. Planktonic cells grown in a liquid system could be directly diluted (using dilution medium at pH 7.4 and 0% (w/v) NaCl) and inoculated in/on the appropriate support. However, surface colonies grown on a gelled surface were first liquefied in a thermostatic water bath at 37°C and homogenized in the stomacher for 30 seconds, before dilution and inoculation in/on the inactivation support. When inactivated in a liquid carrier, the sample was properly diluted (and melted in case of surface colonies grown on gelatin) to obtain a cell density of 5.5 log(CFU/mL). Next, 100 µL was pipetted on empty 5 cm petri dishes, which were closed until CAP treatment. For inactivation on a solid(like) surface, 50 µL of the appropriately diluted sample of either planktonic or colony cells was pipetted and spread on the surface of a gelled medium, at the specific condition, prepared in a 5 cm petri dish (surface area 19.6 cm^2).

This results in a final cell density of 5.5 log(CFU/cm^2) before inactivation. The surface inoculated gelatin medium was allowed to dry in a laminar flow cabinet (Telstar Laboratory Equipment, Woerden, The Netherlands) for 40 min, to ensure treatment of cells on a solid(like) surface instead of treatment in a liquid film on top of the gelled surface. Cell inoculation on a (membrane) filter (cyclopore PC circles, 0.2 µm, diameter 2.5 cm, Whatman, Maidstone, UK) was similar to the aforementioned protocol for solid(like) surfaces, except that the volume pipetted and spread on the filter was 12.5 µL. Regarding the filter surface area of 4.9 cm^2, the final inoculum density was again 5.5 log(CFU/cm^2).

2.5 CAP: equipment and inactivation procedure

A dielectric barrier discharge reactor (similar to that reported by Massines et al., 1998, Figure 2) generated the helium (purity 99.996%) - oxygen (purity ≥ 99.995%) plasma, at flow rates of respectively 4 L/min and 40 mL/min. Samples were placed between the DBD electrodes, with a gap of 0.8 cm, and first the reactor was flushed with the helium-oxygen gas mixture for 4 min, while the current was off. Afterwards, the plasma was generated and samples were treated with CAP for up to 10 minutes (3 min for the filters) at a peak-to-peak voltage around 7 kV, frequency of 15 kHz and dissipated plasma power of 9.6 W. For these experimental conditions the temperature increase of the sample, measured directly after treatment, was about 2°C. An enclosure around the electrode increases the residence time of the plasma species around the sample while also providing a more controlled environment.

2.6 Cell recovery and microbiological analysis

Cell proliferation after CAP treatment was determined via plate counting on both general and selective media. For cells inactivated in a liquid carrier, 900 µL of saline solution (0.85% (w/v) NaCl) was added to the sample. Afterwards, the diluted sample (1 mL) was transferred

to a sterile Eppendorf, in order to prepare serial decimal dilutions. For cells inactivated on the solid(like) surface, the content of the petri dish was transferred to a stomacher bag, liquefied in a thermostatic water bath at 37°C and homogenized in the stomacher for 30 seconds. 1 mL was taken from this bag, and serial decimal dilutions were prepared with saline solution. When inactivated on a filter, the filter was transferred to a stomacher bag containing 5 mL of the saline solution and homogenized in the stomacher for 30 seconds. Again, 1 mL was pipetted from this bag, and appropriate serial decimal dilutions were prepared.

For all samples, 2-4 dilutions were plated (49.2 µL) onto TSA or BHI-Agar plates using a spiral plater (Eddy-Jet, IUL Instruments). In order to determine potential sublethal cell injury, these dilutions were also plated onto XLD-Agar (Xylose Lysine Deoxycholate Agar, Merck & Co, New Jersey, USA) for *S.* Typhimurium and PALCAM-Agar (VWR Chemicals, Leuven, Belgium) for *L. monocytogenes*. Plates with general media were placed at 37°C for 24 h before counting, while selective plates were stored up to 48 h at 30°C. Cell counts shown in the figures are the mean of all countable dilutions for each sample.

2.7 Modelling, parameter estimation and estimation of sublethal injury

Experimental data were fitted with the model of Geeraerd et al. (2000), describing a microbial inactivation curve consisting of a shoulder, a log linear inactivation phase and a tail:

$$\log N(t) = \log\left(\left(10^{\log N_0} - 10^{logN_{res}}\right) \cdot \exp(-k_{max} \cdot t) \cdot \left(\frac{\exp(k_{max}.t_l)}{1+(\exp(k_{max}.t_l)-1)\cdot\exp(-k_{max}\cdot t)}\right) + 10^{logN_{res}}\right) \quad (1)$$

where $N(t)$ [CFU/mL] is the cell density at time t [s], N_0 [CFU/mL] the initial cell density, N_{res} [CFU/mL] a more resistant subpopulation, k_{max} [1/s] the maximum specific inactivation rate and t_l [s] the length of the shoulder. The final *log reduction* is calculated from the difference between log N_0 *and* log N_{res}. In this work, if log N_{res} was not yet reached, log

N(t=600 sec)(*or* log *N(t=180 sec)*, for inactivation on a filter) was used to calculate the log reduction.

Parameters of the Geeraerd et al. (2000) model were estimated via the minimization of the sum of squared errors (SSE), using the *lsqnonlin* routine of the Optimization Toolbox of Matlab (The Mathworks Inc.). Simultaneous with parameter estimation, the parameter estimation errors were determined based on the Jacobian matrix. The Root Mean Squared Error (RMSE) was added as an absolute measure of the goodness of the model fit to the actual observed data.

As treated cells were plated on both general and selective plating media, it was possible to determine the percentage of sublethal injury (SI) as a function of treatment time. The theoretical concentrations obtained from the Geeraerd et al. (2000) model were used to calculate this percentage of sublethal injury (% SI). The percentage of injured survivors after exposure to CAP treatment was determined by means of the following equation (Busch and Donnelly, 1992), providing the extent of the injured population at each exposure time:

$$\%\ \text{Sublethal Injury} = \frac{\text{counts on non selective medium} - \text{counts on selective medium}}{\text{counts on non selective medium}} . 100 \qquad (2)$$

2.8 Statistical analysis

The analysis of variance (ANOVA) test was performed to determine whether there were significant differences amongst means of logarithmically transformed plate counts, at a 95.0% confidence level ($\alpha = 0.05$). The Fisher´s Least Significant Difference (LSD) test was used to distinguish which means were significantly different from which others. Standardized skewness and standardized kurtosis were used to assess if data sets came from normal distributions. These analyses were performed using Statgraphics Centurion XVI.I Package (Statistical Graphics, Washington, USA). Test statistics were regarded as significant when P was ≤ 0.05.

3. RESULTS AND DISCUSSION

Inactivation kinetics of stationary phase *S.* Typhimurium and *L. monocytogenes* cells exposed to CAP treatment are shown in Figure 3 and 4 respectively. For each microorganism, cells were inactivated in a liquid carrier (a), on a solid(like) surface (b) or on a filter (c). Prior to the CAP treatment, cells were grown planktonically or as surface colonies. As a control, cells from the preculture were also treated directly. The experimental data were fitted with the Geeraerd et al. (2000) model. Table 1 and 2 summarize the estimated main inactivation parameters from the Geeraerd model, i.e., the length of the shoulder phase t_l, the inactivation rate k_{max}, the cell density in the tail log N_{res}, the overall *log reduction* and the corresponding statistical analysis.

The CAP mode of action for inactivation of microbial cells may be explained at different levels. Accumulations of charged particles at the surface of the cell membrane induce its rupture. Oxidation of the lipids, amino acids and nucleic acids with reactive oxygen and nitrogen species may cause changes that lead to microbial death or injury. In addition to reactive species, UV photons can modify microbial DNA (Fernandez and Thompson, 2012). As the cell wall and its structure play an important role in the CAP inactivation mechanism, differences can be expected between the inactivation of *S.* Typhimurium (Gram-negative bacteria) and *L. monocytogenes* (Gram-positive bacteria). While both microorganisms have a different cell wall structure, some similarities in their CAP inactivation kinetics are observed (Salton and Kim, 1996). In particular, the shapes of the inactivation kinetics (for each inactivation support) are alike for both microorganisms. However, as often reported, the Gram-positive *L. monocytogenes* is more stress resistant, in this case towards CAP treatment, resulting in lower inactivation efficacies (Jasson et al., 2007; Yuste et al., 2004).

3.1 Effect of growth morphology on the CAP inactivation efficacy

The intrinsic food structure influences the growth morphology of microbial cells. Therefore the growth morphology, i.e., cells grown planktonically or as surface colonies, itself also has an effect on the CAP inactivation efficacy.

3.1.1 Effect of growth morphology for cells inactivated in a liquid carrier

When *S.* Typhimurium is inactivated in a liquid carrier (a), the inactivation kinetics for all growth morphologies have a similar shape: a long shoulder phase, indicating that cells need to be exposed to a certain CAP level before becoming lethally damaged, followed by log linear inactivation. However, the CAP inactivation efficacy is better for planktonic cells as compared to cells grown as surface colonies, as can be deducted from both the inactivation kinetics and the estimated parameters (Figure 3 and Table 1). The inactivation curves for cells studied in a liquid carrier exhibit a shoulder phase, but direct conclusions on the influence of the growth morphology on t_l cannot be drawn, other than $t_{l,\ preculture}$ exhibits the highest value, as (statistical) differences are limited ($P > 0.05$, for most cases). k_{max} values are in most cases similar or slightly higher for planktonic cells. Log N_{res} is undefined since for none of the experiments, inactivated in a liquid carrier, a tailing phase is reached. However, the *log(10) reductions* indicate that higher reductions are obtained for cells grown planktonically.

As for *S.* Typhimurium, *L. monocytogenes* cells inactivated in a liquid carrier (a) exhibit mostly a long shoulder followed by log linear inactivation (Figure 4 and Table 2). However, the inactivation kinetics for cells grown planktonically, only have a long log linear inactivation phase. For all growth morphologies, the overall inactivation is limited, with a maximum *log reduction* around 0.8. Following this, differences between the growth

morphologies are also limited. This translates as well to the inactivation parameters: t_l, k_{max}, and the *log reduction*.

3.1.2 Effect of growth morphology for cells inactivated on a solid(like) surface

For *S.* Typhimurium cells inactivated on a solid(like) surface (b), large differences in inactivation kinetics are present between growth morphologies. The trend of better CAP inactivation efficacies for planktonic cell growth (and cells treated directly from the preculture) is very outspoken in case of inactivation on a solid(like) surface. Values for the length of the shoulder phase are similar ($P > 0.05$). Concerning the inactivation rates the order ($k_{max,\ preculture} \geq$) $k_{max,\ planktonic\ cells} \geq k_{max,\ surface\ colonies}$ is observed. All inactivation kinetics have a tail, indicating the presence of a CAP-resistant population, and log N_{res} is the lowest for cells grown planktonically or treated directly from the preculture. Following these parameters, the *log reductions* are as well lower for cells grown as surface colonies.

Also for *L. monocytogenes* cells inactivated on a solid(like) surface (b), the CAP efficacy is higher for planktonic cells. While no shoulder is observed, a tailing phase is always present and the order (log $N_{res,\ preculture} \leq$) log $N_{res,\ planktonic\ cells} \leq$ log $N_{res,\ surface\ colonies}$ is respected. While no statistical differences ($P > 0.05$) are detected concerning the inactivation rate between the growth morphologies, k_{max} values are the lowest for cells grown as surface colonies. Following this, surface colonies also have the lowest log reductions.

3.1.3 Effect of growth morphology for cells inactivated on a filter

As for *S.* Typhimurium cells inactivated on a filter (c), k_{max} and the *log reductions* are the highest for planktonic cells. Regarding the shoulder phase, t_l values for the growth morphologies are similar ($P > 0.05$).

For *L. monocytogenes* (c), the inactivation kinetics lack a shoulder phase but consist of a log

linear inactivation phase followed by a tail. Concerning the influence of the growth morphology, log N_{max} tends to be the highest for cells grown as surface colonies. No significant differences ($P > 0.05$) between the growth morphologies are detected regarding the inactivation rate k_{max}. The *log reduction* is again the lowest for cells grown as surface colonies.

The lower inactivation efficacy for cells grown as surface colonies suggests that there is an increased resistance towards CAP inactivation for both *S.* Typhimurium and *L. monocytogenes*. When grown in a liquid environment, microorganisms grow planktonically, and a local uniform environment is created due to convective transport of nutrients, metabolites and oxygen (Dens and Van Impe, 2001; McMeekin et al., 2002). On a solid(like) structure, cells are immobilized by the environment forcing them to grow as surface colonies (Brocklehurst et al.,1997; Wilson et al., 2002). Transport in a solid(like) environment is based on slower diffusion, limiting nutrient delivery and metabolite removal, resulting in the formation of concentration gradients around colonies (Antwi et al., 2006; Malakar et al., 2000; Wimpenny and Coombs, 1983). The solid(like) environment results in an additional stress for the colonies. However, the static experimental set-up during the growth phase prior to inactivation, results in similar growth rates for both growth morphologies (Smet et al., 2015a, 2015b). Still, nutrient limitation in case of surface colonies could lead to starvation stress, i.e., the survival of bacteria in oligotrophic conditions (i.e., an environment with sufficient oxygen but limited nutrient availability) (Wesche et al., 2009). This starvation stress might be less important during the initial growth phase but might promote resistance to subsequent stresses, like CAP treatment. Archer (1996) reported on the importance of carbon-limitation-induced genes in the protection of *S.* Typhimurium from starvation. It was noted that these genes could induce a system of cross-protection against

other stresses such as thermal, osmotic or oxidative stress. Cross-protection due to starvation stress could be a reason for the observed increased resistance to plasma treatment for cells grown as surface colonies (Li et al., 2013). Additionally, under stressing conditions the influence of the solid microstructure itself becomes more important. As reported for growth in Smet et al. (2015a, 2015b), while for most environmental conditions μ_{max} values of planktonic cells and surface colonies show no significant differences under static conditions, this observation is not valid for very stressing environmental conditions. For example, at very low temperatures or high osmotic stresses, μ_{max} values for both growth morphologies are no longer similar and planktonic cells exhibit higher growth rates as compared to surface colonies. As CAP also induces stress on the microorganisms, again the microstructure can be expected to have an important effect on the inactivation behavior.

3.2 Effect of inactivation support system on CAP inactivation efficacy

Next to the influence of the growth morphology, also the effect of the food structure was evaluated by using different support systems during CAP treatment: cells were inactivated in a liquid carrier (a), on a solid(like) carrier (b) or on a filter (c) (Figure 3 and 4, Table 1 and 2). While the previous section discusses the influence of the growth morphology on the CAP efficacy for cells inactivated on one specific support system, this section describes and compares the inactivation kinetics between the different support systems.

When inactivated in a liquid carrier (a), the inactivation curves for both *S.* Typhimurium and *L. monocytogenes* have a long shoulder phase, which is followed by the log linear inactivation phase, and no tailing phase is observed. Cells treated on a solid(like) surface (b), follow different inactivation kinetics. For both microorganisms, there is no shoulder (or only a very short one) and the log linear inactivation phase is followed by a tailing phase. Finally, for inactivation on a filter (c), no general trend is observed. Survival curves for *S.* Typhimurium

consist of a short shoulder phase followed by the log linear inactivation phase. Regarding *L. monocytogenes*, the log linear inactivation is followed by a long tail.

Since parameters for log N_0 and log N_{res} are expressed with different units depending on the inactivation support (log(CFU/cm^2) for the solid(like) surface and filter, and log(CFU/mL) for the liquid carrier), it is not possible to directly compare these inactivation parameters for the different systems. Nevertheless, all other parameters estimated with the Geeraerd et al. (2000) model, t_l, k_{max} and the *log reduction* can be compared between the different support systems used.

As *S.* Typhimurium cells inactivated in a liquid carrier (a) exhibit a very long shoulder, this results in high values for the length of this shoulder as compared to t_l values for cells inactivated on a solid(like) surface (b) or a filter (c) ($P \leq 0.05$). Inactivation rates for cells studied in a liquid carrier (a) are lower as compared to the other inactivation support systems. Regarding the *log reductions*, no specific trend is present, as for each of the three inactivation support systems a broad range of *log reductions* (1.0-2.9) is observed.

For *L. monocytogenes* a shoulder phase is only observed for cells inactivated in a liquid carrier (a), thus no conclusions can be drawn regarding the parameter t_l. Inactivation rates and *log reductions* for cells inactivated in a liquid carrier (a) are again significantly lower as compared to the other inactivation support systems ($P \leq 0.05$). *Log reductions* for cells inactivated in a liquid carrier (a) range from 0.2 to 0.8, which is lower than reductions for the other support systems (on a solid(like) surface (b): 0.9-2.2, on a filter (c): 1.5-1.9).

In general, the main difference between the inactivation supports is presence or absence of a shoulder. If cells are inactivated in the liquid carrier, a very long shoulder is present, while for cells inactivated on a solid(like) surface or a filter, the shoulder is very short or even absent.

In case of inactivation on a solid(like) surface or a filter, this absence leads to a very rapid and efficient inactivation.

The structure of the support has an influence on the CAP inactivation efficacy. Noriega et al. (2011) reported significant differences in CAP efficacy for *Listeria innocua* inactivation on surfaces with different topographies, i.e., the disinfection of a membrane filter surface, chicken skin or chicken meat. As in the present study, the smooth and dense fiber network of the membrane filter prevents possible cell migration (into the gelled media below). For other surfaces, e.g. gelled media, it has been reported that bacteria deposited at the surface could be drawn through capillary action into surface irregularities (Kim et al., 1996). The smooth surface of the filters, with the cells directly exposed to the plasma, explains the shorter treatment times needed for cells inactivated on a filter.

Similar to the reduced inactivation efficacy that was observed for cells inactivated in a liquid carrier as compared to treatment of cells on a solid(like) surface or on a filter, Laroussi et al. (2000) observed longer treatment times and lower log reduction for CAP inactivation of *E. coli* in a liquid broth compared to cells inactivated on a filter. This difference could be attributed to the reduced potential of the CAP active components to penetrate and diffuse in the liquid broth where the microbial cells are dispersed freely. As many highly reactive plasma species react at the plasma-liquid interface and do not penetrate very far into the liquid, the effectiveness of the CAP inactivation in a liquid carrier is reduced. Cells treated on a solid(like) surface or a filter surface are more easily attained by these active CAP components during the treatment (Oehmigen et al., 2010). Additionally, at the initial cell load of $10^{5.5}$ CFU/cm^2 the bacterial cells are arranged in a monolayer on the filter or solid(like) surface, and thus no protection against CAP treatment is provided by other cells (Bayliss et al., 2012). Fernandez et al. (2012) studied the effect of the microbial load on the efficacy of CAP inactivation of *S.* Typhimurium. It was concluded that at higher initial cell loads, i.e.

starting from 10^7 CFU/filter ($\approx 2.10^6$ CFU/cm^2), multilayers of cells are formed on a filter surface, protecting cells below.

As a remark, it should be mentioned that after a certain treatment time ($\geq$ 5 min), the small liquid volume of 100 µL was significantly dried by the gas plasma. The evaporation of the liquid was studied as a function of treatment time, and the evaporation rate determined was 0.0087 ± 0.0006 g/sec. The evaporation possibly results in a shift of treatment of cells in a liquid carrier towards cell treatment on a solid surface (i.e., the plastic bottom of the petri dish) during long treatment times. It also contributes to the fact that inactivation of *S.* Typhimurium and *L. monocytogenes* cells inactivated in a liquid, mainly starts at longer treatment times. To confirm that the sample drying out was not due to the gas flow itself, as well that no cells were swept along by this flow, tests were executed without any current (only gas flow). In these control tests, the volume of the sample (i.e., determined by the mass of the sample) and the cell density remained intact. This indicates that the drying out of samples and the decrease in cell density are indeed due to the CAP treatment.

3.3 CAP and sublethal injury of cells

To investigate a potential sublethal injury of cells treated with CAP, treated cells were plated on both general and selective plating media. Figure 5 and 6 show the percentage of sublethal injury (SI) as a function of time. For *S.* Typhimurium in general the percentage of sublethal injury is high, with values up to 90%, while SI is lower for *L. monocytogenes*. Differences between the two microorganisms, together with the influence of the food structure on the SI of cells treated with CAP, will be discussed in this section. Next to this, the relation of the SI evolution to the inactivation kinetics will be described.

3.3.1 CAP and SI of cells: evolution of SI and relation to inactivation kinetics

For most of the curves, sublethal damage increases in time, and after reaching a maximum value, decreases again. The point of the maximum level of sublethal injury coincides with the start of a new phase on the inactivation curve. Some general trends for each microorganism and support system can be observed as will be discussed below.

For *S.* Typhimurium cells inactivated in a liquid carrier (a), the inactivation curves show a shoulder phase followed by log linear inactivation. For cells treated directly from the preculture, the maximum SI coincides with the start of the log linear inactivation phase. Regarding the other growth morphologies, no maximum was observed for the SI evolution. *L. monocytogenes* cells for this inactivation support system (a) also do not present a maximum in the evolution of the SI.

When *S.* Typhimurium cells are inactivated on a solid(like) surface (b), inactivation curves have a shoulder phase, a log linear inactivation phase and a tail. The maximum SI coincides with the transfer from the shoulder to the log linear inactivation phase. A second bend present in the SI evolution, corresponds to the start of the tail. During the tailing phase, the percentage of SI remains constant or is equal to zero. In case of *L. monocytogenes* inactivated on a solid(like) surface (b), the maximum SI indicates the transition to the tail during which the SI becomes constant. If also a shoulder is present (cells from the preculture), the maximum indicates the start of the log linear inactivation, and a second bend the transition towards the tailing phase.

As well for *S.* Typhimurium and *L. monocytogenes* cells inactivated on a filter (c), the maximum SI equals the start of a new phase on the inactivation curves.

The rapid increase of SI with exposure time to CAP, and after reaching a maximum peak, followed by a decrease suggests a mechanism of injury accumulation that culminates in cell death (Noriega et al., 2013). This same phenomenon was observed by Perni et al. (2007b),

where *E. coli* and *S.* Typhimurium were exposed to nanosecond pulsed electric fields (PEF) and a similar threshold of cell damage, followed by cell death was observed. As stated in Rowan et al. (2007), knowledge concerning SI caused by CAP treatment is limited. However, the analogy between CAP and PEF regarding their actions on the cell membrane, can explain the observed similarities concerning SI (Misra et al., 2011).

In some other cases no maximum is observed, and the percentage of sublethally injured cells is still increasing at the maximum CAP treatment time. For *S.* Typhimurium cells, this trend is observed for both planktonic cells and cells grown as surface colonies when inactivated in a liquid carrier. SI is also still increasing at the maximum treatment time for cells directly treated from the preculture which are inactivated on a filter. Regarding *L. monocytogenes*, the same observations are made for cells treated directly from the preculture or grown as surface colonies which are inactivated in the liquid carrier. This trend corresponds to experimental conditions where no tailing phase is yet reached for the maximum treatment time, and further inactivation might still be possible if the treatment times would be extended.
Finally, for *L. monocytogenes* cells grown planktonically and inactivated in a liquid carrier (a), there is no SI detected. In this specific case, the total log reduction remains very limited as well.

3.3.2 CAP and SI of cells: effect of the food structure

The previous section describes some general trends regarding the SI evolution with treatment time. The general trends discussed above are found for all inactivation support systems. This indicates that the system itself, and thus the food structure does not especially influence the SI evolution. Considering the different growth morphologies, no clear trends are present explaining the influence of CAP treatment on the SI. In most cases, the percentage of SI for

cells grown as surface colonies tends to be the lowest.

4. CONCLUSION

This work studied the influence of the food structure on the potential of CAP to inactivate *S.* Typhimurium and *L. monocytogenes*. Next to the effect of the cell growth morphology, the impact of the food structure during inactivation on the CAP efficacy was investigated. Although *L. monocytogenes* is more resistant to treatment as compared to *S.* Typhimurium, similar trends regarding the influence the food structure on the CAP inactivation efficacy are detected. Cell immobilization, for cells grown as surface colonies, often results in more resistant cells during CAP treatment. Cross-protection due to starvation stress can explain this increased resistance to plasma treatment for treatment of both microorganisms. Regarding the food structure during the inactivation, cells in a liquid carrier are more difficult to inactivate as compared to cells inactivated on a solid(like) surface, the CAP active species have to diffuse throughout the liquid medium. As many highly reactive plasma species react at the plasma-liquid interface and do not penetrate very far into the liquid, the effectiveness of the CAP inactivation in a liquid carrier is limited. The SI evolution is not really influenced by the growth morphology or the inactivation support. Often a maximum in the SI evolution is present, indicating an injury accumulation culminating in cell death. This research demonstrates that the food structure significantly influences the inactivation behavior and efficacy of CAP. This indicates that the food structure needs to be taken into account when designing treatment of food products with CAP in order to ensure efficient treatment, resulting in safer food products. Future studies should focus on the influence of food intrinsic factors in general, as not only the food structure might have a major impact on the CAP efficacy. Next to microbial studies, the possible influence of CAP on the food quality is important. Quality parameters (e.g., vitamins, pH (Shi et al., 2011)) need to be assessed and

combined with microbial studies to better optimize the process. Finally, as the CAP technology evolves in time, this will also influence its cost aspect. This is mainly determined by the amount of electricity required together with the choice of feed gas (Niemira, 2012). Further research and improvements will make it (economically) feasible to implement the CAP technology in the food industry.

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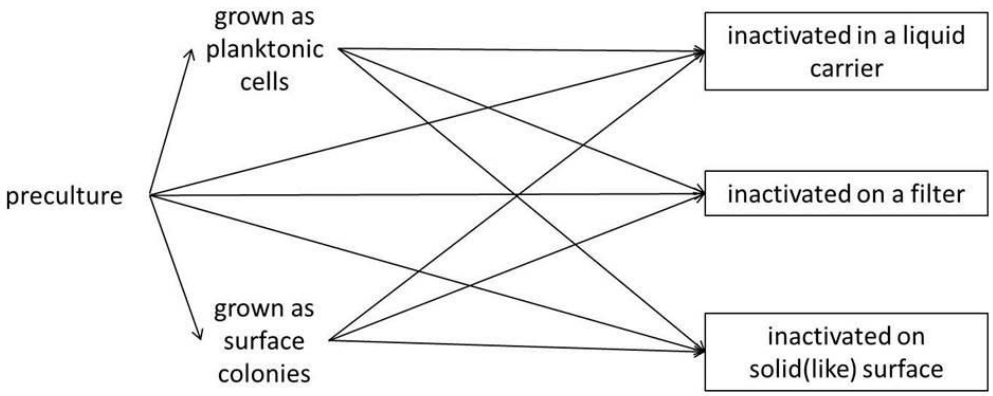
a)



b)

Growth morphology	Inactivation support	Example
Planktonic cells	Liquid carrier	- Microorganisms have grown in the liquid food product, which is treated with CAP - Microorganisms have grown in water that is added to the (liquid) food product, which is treated with CAP
Surface colonies	Solid(like) surface	- Microorganisms have grown on the surface of the food product, which is treated with CAP - Microorganisms have grown on knife used to cut the food product (or have grown on another surface in contact with the food product) and are transferred to the surface of the food product, which is treated with CAP
Surface colonies	Liquid carrier	- Microorganisms have grown on the surfaces of the food product, which was blended into a liquid food product, and treated with CAP - Microorganisms have grown inside blender/ on knife used to liquefy the food product (or have grown on another surface in contact with the food product), and the liquefied food product is treated with CAP
Planktonic cells	Solid(like) surface	- Microorganisms have grown in washing water to remove dirt from the food product and get transferred to the food surface, which is treated with CAP

Figure 1: (a) Different combinations tested at each experimental condition. Cells were inactivated in a liquid carrier, on a solid(like) surface and on a filter. Prior to CAP treatment, cells were grown planktonically or as surface colonies. As a control, cells from the preculture were directly treated and filters were added as inactivation support system.
(b) Examples for the different combinations.

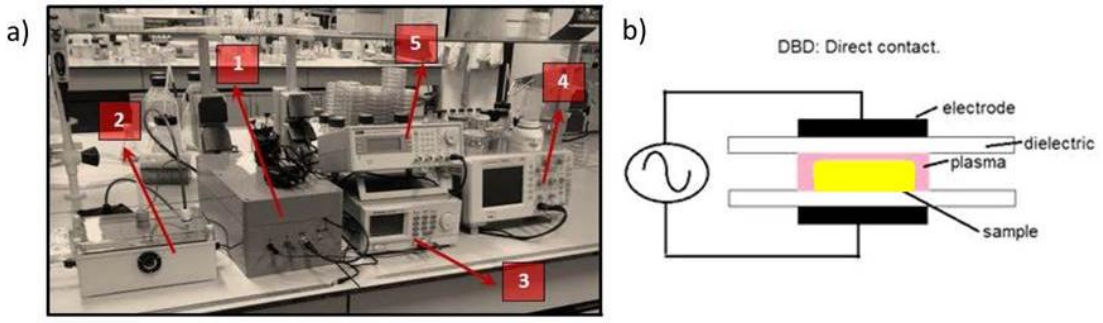


Figure 2: (a) the CAP set up: (1) plasma power source, (2) Dielectric Barrier Discharge reactor, (3) DC power supply, (4) oscilloscope and (5) function generator. (b) DBD electrode inside reactor (Massines et al., 1998).

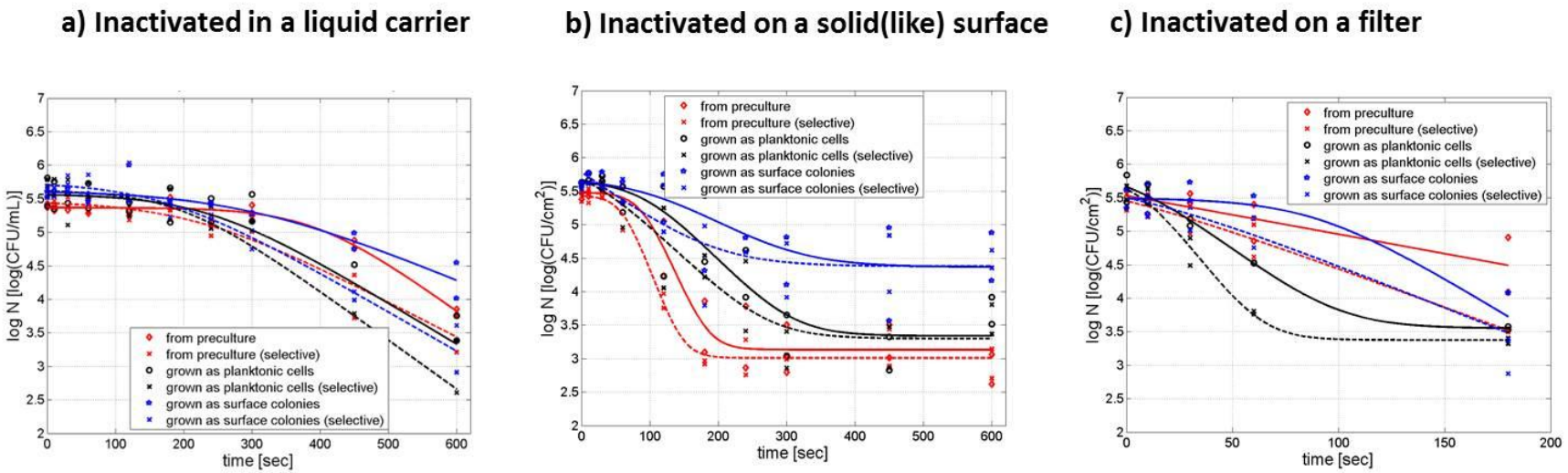


Figure 3: Survival curves of stationary phase *S.* Typhimurium after exposure to CAP. Cells were inactivated in a liquid carrier (a), on a solid(like) surface (b) and on a filter (c). Prior to CAP treatment, cells were grown either planktonically or as surface colonies. As a control, cells from the preculture were directly treated. Experimental data (symbols) and global fit (line) of the Geeraerd et al. (2000) model: total viable population (o, solid line) and uninjured viable population (x, dashed line).

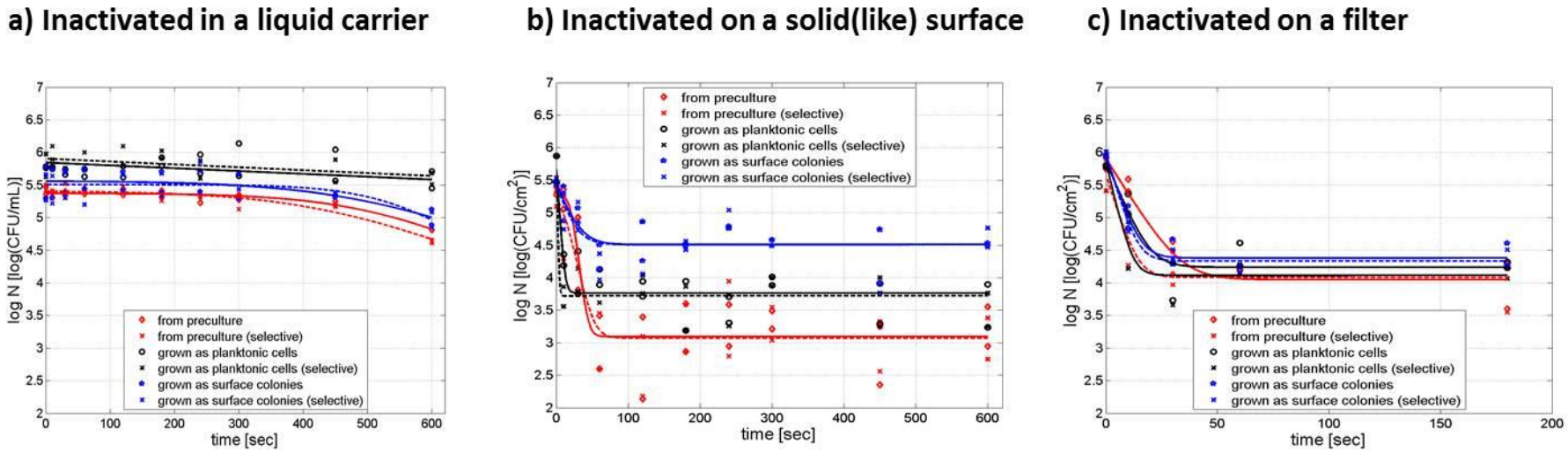


Figure 4: Survival curves of stationary phase *L. monocytogenes* after exposure to CAP. Cells were inactivated in a liquid carrier (a), on a solid(like) surface (b) and on a filter (c). Prior to CAP treatment, cells were grown planktonically or as surface colonies. As a control, cells from the preculture were directly treated. Experimental data (symbols) and global fit (line) of the Geeraerd et al. (2000) model: total viable population (o, solid line) and uninjured viable population (x, dashed line).

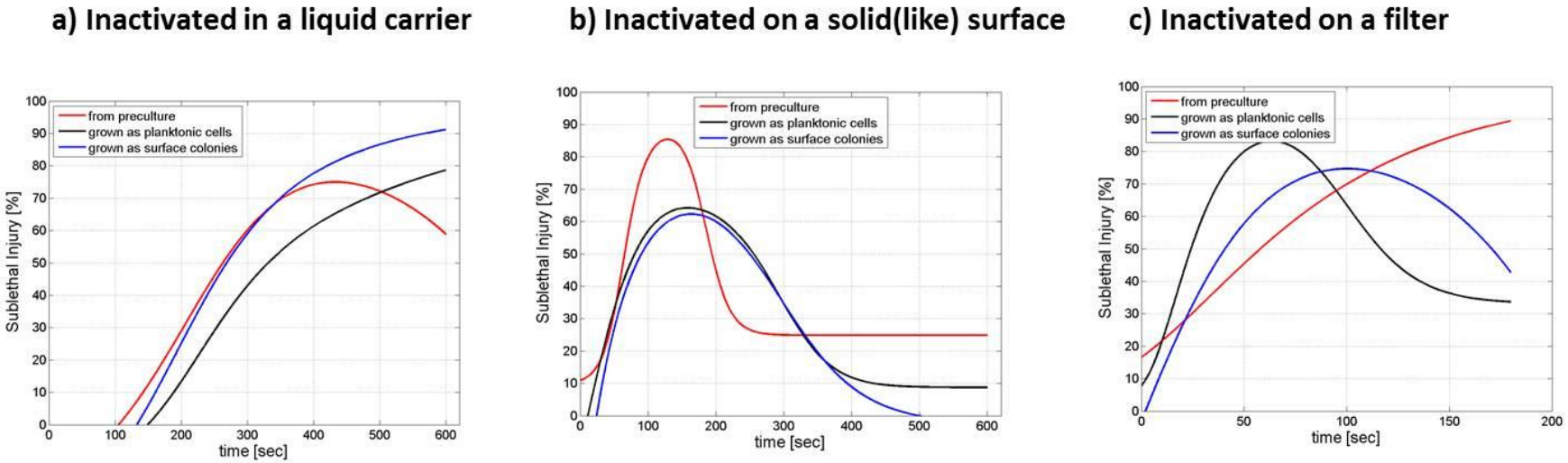


Figure 5: Evolution with time of the sublethal injury (%) of *S.* Typhimurium towards the exposure time to CAP. Cells were inactivated as a liquid (a), on a solid(like) surface (b) and on a filter (c). Prior to CAP treatment, cells were grown planktonically or as surface colonies. As a control, cells from the preculture were directly treated.

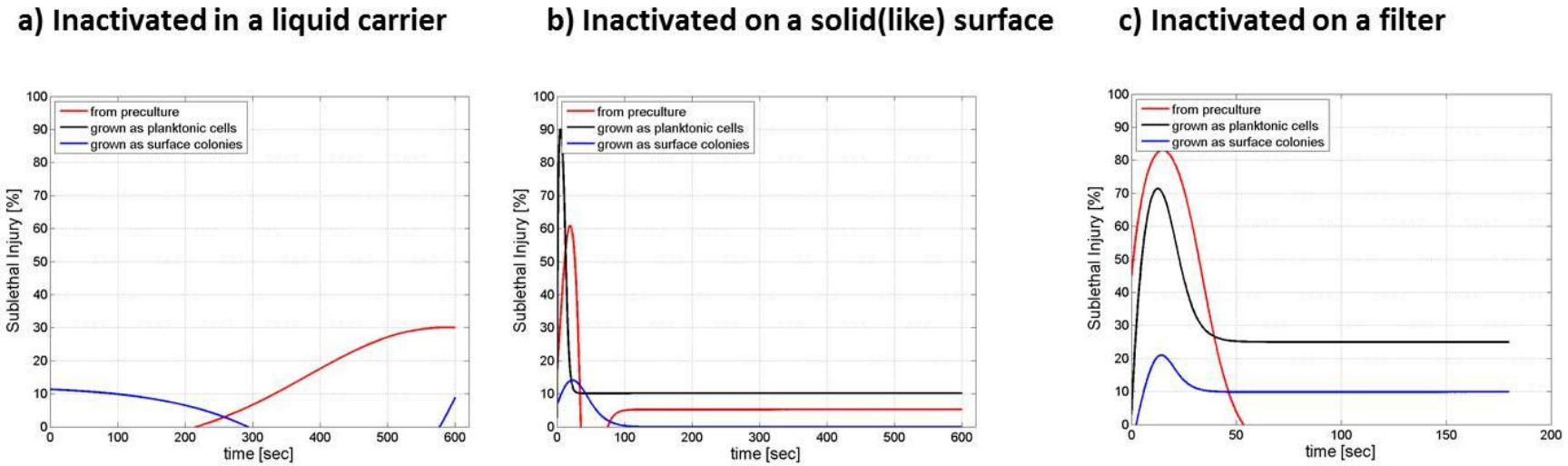


Figure 6: Evolution with time of the sublethal injury (%) of *L. monocytogenes* towards the exposure time to CAP. Cells were inactivated as a liquid (a), on a solid(like) surface (b) and on a filter (c). Prior to CAP treatment, cells were grown planktonically or as surface colonies. As a control, cells from the preculture were directly treated.

Table 1. Inactivation parameters of the Geeraerd et al. (2000) model for *S.* Typhimurium after exposure to CAP. Cells were inactivated on a liquid carrier, on a solid(like) surface and on a filter. Prior to CAP treatment, cells were grown planktonically, as surface colonies, or were treated directly from the preculture.

Support system	Reference	Growth morphology	Population	Inactivation parameters				RMSE	$_1$log reduction$_2$
				$_1$log N0 (log (CFU/mL))$_2$ / $_1$log N0 (log(CFU/cm^2))$_2$	$_1t_l$ (s)$_2$	$_1k_{max}$ (1/s)$_2$	$_1$log N_{res} (log(CFU/mL))$_2$ / $_1$logN_{res} (log(CFU/cm^2))$_2$		
liquid	a	Preculture	Total	$5.4 \pm 0.0_A$	$_{(b)}392.2 \pm 21.1_B$	$_{(a)}0.017 \pm 0.002_B$	-	0.1122	$\approx {}_{(b)}1.6 \pm 0.0_B$
			Uninjured	$5.4 \pm 0.1_A$	$_{(b)}220.6 \pm 41.5_A$	$_{(a)}0.012 \pm 0.002_A$	-	0.1996	$\approx {}_{(a)}2.0 \pm 0.1_A$
		Planktonic cells	Total	$5.6 \pm 0.1_B$	$_{(b)}238.3 \pm 74.3_A$	$_{(a)}0.014 \pm 0.003_{AB}$	-	0.4204	$\approx {}_{(a)}2.3 \pm 0.1_C$
			Uninjured	$5.6 \pm 0.2_{AB}$	$_{(b)}196.8 \pm 58.7_A$	$_{(a)}0.017 \pm 0.003_B$	-	0.4567	$\approx {}_{(b)}2.9 \pm 0.2_C$
		Surface colonies	Total	$5.6 \pm 0.1_B$	$_{(b)}296.8 \pm 47.5_{AB}$	$_{(a)}0.010 \pm 0.002_A$	-	0.1567	$\approx {}_{(a)}1.3 \pm 0.1_A$
			Uninjured	$5.7 \pm 0.1_B$	$177.4 \pm 34.9_A$	$_{(a)}0.014 \pm 0.001_{AB}$	-	0.2061	$\approx {}_{(c)}2.5 \pm 0.1_B$
solid(like) surface	b	Preculture	Total	$5.5 \pm 0.1_A$	$_{(a)}88.9 \pm 21.4_A$	$_{(b)}0.057 \pm 0.019_B$	$3.1 \pm 0.1_A$	0.3386	$_{(c)}2.4 \pm 0.1_B$
			Uninjured	$5.4 \pm 0.1_A$	$_{(a)}61.8 \pm 12.9_A$	$_{(b)}0.065 \pm 0.014_B$	$3.0 \pm 0.1_A$	0.1970	$_{(b)}2.4 \pm 0.1_B$
		Planktonic cells	Total	$5.6 \pm 0.2_A$	$_{(a)}95.8 \pm 54.3_A$	$_{(a)}0.026 \pm 0.010_A$	$3.3 \pm 0.2_A$	0.4455	$_{(a)}2.3 \pm 0.3_B$
			Uninjured	$5.7 \pm 0.2_{AB}$	$_{(a)}33.0 \pm 57.6_A$	$_{(a)}0.023 \pm 0.008_A$	$3.3 \pm 0.2_A$	0.3842	$_{(a)}2.4 \pm 0.3_B$
		Surface colonies	Total	$5.6 \pm 0.2_A$	$_{(a)}117.1 \pm 113.5_A$	$_{(ab)}0.017 \pm 0.015_A$	$4.4 \pm 0.3_B$	0.4549	$_{(a)}1.2 \pm 0.4_A$
			Uninjured	$5.8 \pm 0.2_B$	-	$_{(a)}0.017 \pm 0.005_A$	$4.4 \pm 0.2_B$	0.3666	$_{(a)}1.4 \pm 0.3_A$
filter	c	Preculture	Total	$5.5 \pm 0.2_A$	$_{(a)}2.2 \pm 163.3_A$	$_{(a)}0.014 \pm 0.011_A$	-	0.2917	$\approx {}_{(a)}1.0 \pm 0.2_A$
			Uninjured	$5.5 \pm 0.1_A$	$_{(a)}13.6 \pm 32.6_A$	$_{(a)}0.027 \pm 0.005_A$	-	0.2149	$\approx {}_{(a)}2.0 \pm 0.1_A$
		Planktonic cells	Total	$5.7 \pm 0.1_A$	$_{(a)}12.4 \pm 15.1_A$	$_{(b)}0.057 \pm 0.017_B$	3.5 ± 0.1	0.1482	$_{(a)}2.2 \pm 0.1_C$
			Uninjured	$5.6 \pm 0.1_A$	$_{(a)}10.1 \pm 7.6_A$	$_{(b)}0.097 \pm 0.019_B$	3.4 ± 0.1	0.1699	$_{(a)}2.2 \pm 0.1_A$
		Surface colonies	Total	$5.5 \pm 0.1_A$	$_{(a)}89.0 \pm 47.6_A$	$_{(b)}0.045 \pm 0.022_{AB}$	-	0.2613	$\approx {}_{(b)}1.8 \pm 0.1_B$
			Uninjured	$5.5 \pm 0.2_A$	$20.1 \pm 52.6_A$	$_{(b)}0.029 \pm 0.009_A$	-	0.3880	$\approx {}_{(b)}2.0 \pm 0.2_A$

[1] For each growth morphology and population type, parameters of the Geeraerd model bearing different subscripts (no lowercase letters in common) are significantly different ($P \leq 0.05$)

[2] For each inactivation support and population type, parameters of the Geeraerd model bearing different subscripts (no uppercase letters in common) are significantly different ($P \leq 0.05$)

Table 2. Inactivation parameters of the Geeraerd et al. (2002) model for *L. monocytogenes* after exposure to CAP. Cells were inactivated on a liquid carrier, on a solid(like) surface and on a filter. Prior to CAP treatment, cells were grown planktonically, as surface colonies, or were treated directly from the preculture.

Support system	Reference	Growth morphology	Population	Kinetic parameters				RMSE	$_1$log reduction$_2$
				$_1$log N0 (log (CFU/mL))$_2$ / $_1$log N0 (log(CFU/cm^2))$_2$	$_1t_l$ (s)$_2$	$_1k_{max}$ (1/s)$_2$	$_1$log N_{res} (log(CFU/mL))$_2$ / $_1$logN_{res} (log(CFU/cm^2))$_2$		
liquid	a	Preculture	Total	$5.4 \pm 0.0_A$	511.9 ± 18.0	$_{(a)}0.011 \pm 0.002_B$	-	0.0557	$\approx {}_{(a)}0.6 \pm 0.0_B$
			Uninjured	$5.4 \pm 0.0_A$	438.0 ± 30.5	$_{(a)}0.010 \pm 0.002_A$	-	0.0822	$\approx {}_{(a)}0.8 \pm 0.0_C$
		Planktonic cells	Total	$5.8 \pm 0.1_C$	-	$_{(a)}0.001 \pm 0.000_A$	-	0.1818	$\approx {}_{(a)}0.2 \pm 0.1_A$
			Uninjured	$5.9 \pm 0.1_B$	-	$_{(a)}0.001 \pm 0.000_A$	-	0.1633	$\approx {}_{(a)}0.3 \pm 0.1_A$
		Surface colonies	Total	$5.6 \pm 0.1_B$	493.0 ± 70.0	$_{(a)}0.009 \pm 0.005_B$	-	0.1840	$\approx {}_{(a)}0.6 \pm 0.1_B$
			Uninjured	$5.5 \pm 0.1_A$	540.0 ± 53.5	$_{(a)}0.015 \pm 0.012_A$	-	0.2031	$\approx {}_{(a)}0.5 \pm 0.1_B$
solid(like) surface	b	Preculture	Total	$5.3 \pm 0.3_A$	20.5 ± 27.6	$_{(a)}0.219 \pm 0.576_A$	$3.1 \pm 0.1_A$	0.4751	$_{(b)}2.2 \pm 0.3_B$
			Uninjured	$5.2 \pm 0.3_A$	9.8 ± 19.5	$_{(ab)}0.119 \pm 0.088_A$	$3.1 \pm 0.1_A$	0.5108	$_{(c)}2.1 \pm 0.3_B$
		Planktonic cells	Total	$5.7 \pm 0.2_A$	-	$_{(c)}0.355 \pm 0.096_A$	$3.8 \pm 0.1_B$	0.3335	$_{(b)}1.9 \pm 0.2_B$
			Uninjured	$5.7 \pm 0.2_B$	-	$_{(a)}1.000 \pm 13.908_A$	$3.7 \pm 0.1_B$	0.3103	$_{(b)}2.0 \pm 0.2_B$
		Surface colonies	Total	$5.5 \pm 0.2_A$	-	$_{(a)}0.070 \pm 0.031_A$	$4.5 \pm 0.1_C$	0.2594	$_{(b)}1.0 \pm 0.2_A$
			Uninjured	$5.4 \pm 0.2_{AB}$	-	$_{(a)}0.077 \pm 0.048_A$	$4.5 \pm 0.1_C$	0.3503	$_{(b)}0.9 \pm 0.2_A$
filter	c	Preculture	Total	$5.9 \pm 0.2_A$	-	$_{(a)}0.127 \pm 0.030_A$	$4.0 \pm 0.1_A$	0.2721	$_{(b)}1.9 \pm 0.2_B$
			Uninjured	$5.6 \pm 0.3_A$	-	$_{(b)}0.252 \pm 0.100_A$	$4.0 \pm 0.1_A$	0.3586	$_{(b)}1.6 \pm 0.3_A$
		Planktonic cells	Total	$5.9 \pm 0.2_A$	-	$_{(b)}0.190 \pm 0.067_{AB}$	$4.2 \pm 0.1_B$	0.2804	$_{(b)}1.7 \pm 0.2_{AB}$
			Uninjured	$5.8 \pm 0.2_A$	-	$_{(a)}0.333 \pm 0.085_A$	$4.1 \pm 0.1_A$	0.2820	$_{(b)}1.7 \pm 0.2_A$
		Surface colonies	Total	$5.9 \pm 0.1_A$	-	$_{(b)}0.235 \pm 0.051_B$	$4.4 \pm 0.1_C$	0.1876	$_{(c)}1.5 \pm 0.1_A$
			Uninjured	$6.0 \pm 0.1_A$	-	$_{(b)}0.265 \pm 0.042_A$	$4.3 \pm 0.1_B$	0.1516	$_{(c)}1.7 \pm 0.1_A$

[1] For each growth morphology and population type, parameters of the Geeraerd model bearing different subscripts (no lowercase letters in common) are significantly different ($P \leq 0.05$)

[2] For each inactivation support and population type, parameters of the Geeraerd model bearing different subscripts (no uppercase letters in common) are significantly different ($P \leq 0.05$)