**Combined effect of CAP, intrinsic and extrinsic factors on the microbial behavior and physicochemical/ rheological parameters of food model systems during storage**

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# ABSTRACT

Microbial decontamination by means of cold atmospheric plasma (CAP) offers great potential for treatment of heat-sensitive food products, extending their storage life. CAP is created by applying a high voltage to a gas stream, resulting in microbial inactivation according to different mechanisms. This paper thoroughly assesses the influence of CAP on the storage life of food model systems inoculated with *Salmonella* Typhimurium, while considering its impact on the product properties. Food model systems, with varying intrinsic factors (pH, salt concentration and food (micro)structure), are treated for 5 minutes using a dielectric barrier discharge reactor generating a helium-oxygen plasma and stored at 8°C or 20°C. During storage, cell densities are determined. Data are fitted with predictive (growth or inactivation) models. CAP treatment prolongs food storage however its rate of success is dependent on both extrinsic and intrinsic factors. An important factor is the storage temperature, as recovery of CAP treated cells proves more difficult when stored at 8˚C. At 20°C, cell growth is merely slowed down. Additionally, at pH 5.5, 6% (w/v) NaCl, osmotic stress is induced on the microorganisms, which results in low cell recovery or further inactivation. The influence of the food structure on the storage behavior is insignificant. Finally, the effect of CAP on these intrinsic factors is determined. pH values and aw measurements indicate a negligible effect of CAP on these product properties, while stress and frequency sweeps indicate a limited influence of CAP treatment on the (micro)structure.

# IMPORTANCE

Although being a very promising technology, most studies regarding the use of cold atmospheric plasma for food decontamination focus on the inactivation of a target microorganism, in relation to a specific food product. Fundamental knowledge on this non-thermal technology, including its impact on the storage life, is lacking. This study investigates the effect of CAP on the microbial behavior during storage. By performing tests on model systems, for a variation of intrinsic and extrinsic factors, this work renders information on the suitability of this novel technology regarding treatment of a broad spectrum of food products. Moreover, this study demonstrates the limited impact of CAP on the food properties, enhancing the suitability of the technology to be implemented in the food industry.

# INTRODUCTION

The use of cold atmospheric plasma (CAP) represents a novel technology with high potential for decontamination of foods products for which traditional technologies fail. The microbiological safety of heat sensitive products, like fruits and vegetables, is often ensured by the use of a washing treatment in combination with chemical biocides (1, 2). The high water consumption together with the possible formation of carcinogenic halogenated by-product, constitute a major disadvantage regarding the use of this technique. Therefore, new innovative and sustainable physical technologies, like CAP, are a good alternative to treat heat sensitive products.

CAP is produced by the excitation of a gas stream with a high voltage, at room temperature and under atmospheric pressure. This excitation results in a mixture of electrons, ions, atomic species, free radicals and UV photons (3, 4). Variable inactivation mechanisms result in cell death. Reactive species and charged particles accumulate at the surface of the cell membrane and bombard it, inducing lesions. Also, (toxic) reactive species diffuse through cell membranes, or penetrate through lesions, and interact with macromolecules, causing local damage. In addition, UV photons (200-300 nm) modify the DNA of the microorganisms by inducing the formation of thymine dimers (5, 6, 7).

The main benefits of CAP treatment are the low temperatures, short treatment times and the fact that no toxic residues are left on food products (8). However, knowledge regarding the impact on the food quality and product properties needs to be further researched (9).

The CAP efficacy, together with its impact on the storage life, depends on several process parameters. Next to the CAP set-up, plasma characteristics (like power, frequency, voltage, gas flow or composition) are of major importance (5, 10, 11, 12). Additionally, the target microorganism, the microbial load and growth phase have an effect on the CAP treatment (13, 14). Finally, it was recently proved that also the (food) sample properties determine the success of the treatment. Firstly, the topography of the sample influences CAP treatment (15). Secondly, factors intrinsic to the sample (e.g., food microstructure, saline concentration, pH) can exert stresses on the microorganisms and impact the efficacy of CAP treatment (16, 17, 18, 19).

For microorganisms, stress includes all conditions deviating from their optimal growth conditions, and can lead to damaging the cell or even cell death. Stress resistanceis related to the possibility of cells to handle these stress factors after exposure. Additionally, cross protection can arise, as cells exposed to a certain type of stress can gain resistance towards subsequent stresses (20). Microorganisms can suffer from stress during different stages in the food production chain: (i) when they survive in an environment where a limited number of nutrients are present, before they contaminate the food product (e.g., survival in water or in equipment), (ii) when surviving on a food product with intrinsic factors far from the optimal for growth (e.g., suboptimal pH, low aw), (iii) during treatment of the product (e.g. pasteurization, HHP, CAP), and (iv) during storage (e.g., low storage temperatures).

The objective of this work is to assess the influence of CAP on the storage life of a food model system, based on the microbial behavior of *S.* Typhimurium CAP at different storage temperatures (optimal or stressing). Survival or growth during the storage is characterized and compared with microbial dynamics of untreated cells. The impact of osmotic stress, suboptimal pH, and food structure are incorporated in the experimental plan. Additionally, the influence of CAP on the model system properties is investigated, directly after treatment and at the end of the storage period.

# MATERIALS AND METHODS

## Influence of CAP during storage

The influence of CAP during storage at different temperatures was studied, incorporating the influence of intrinsic factors (pH, salt concentration, food structure) of the model systems used. *Salmonella* Typhimurium inoculated model systems were stored at two different temperatures. In summary, CAP treated samples were stored, together with untreated controls, at either 20°C for 10 days (mimicking storage at room temperature) or at refrigeration temperature (8°C) for one month. 8°C was selected so that *S.*Typhimurium cells were still able to survive / grow. Cells were grown and stored at pH 7.4 and 0% (w/v) NaCl or pH 5.5 and 6% (w/v) NaCl, an optimal and a very stressing growth condition respectively. The food structure was incorporated as an additional intrinsic factor: (i) during growth expressed as the cells growth morphology (planktonic cells or surface colonies) or, (ii) during treatment and subsequent storage represented by the support structure (liquid carrier or solid(like) surface). Different combinations regarding the food structure, thus growth morphology and support system, were investigated, mimicking different processing steps in the food industry (18). For example, cells grown as surface colonies were subsequently CAP inactivated in a liquid carrier, mimicking the blending of contaminated solid(like) food products into a liquid product that is treated with CAP. Taking into account all different intrinsic and extrinsic factors, this resulted in 16 experimental conditions. All experiments were conducted in duplicate. All these studies are presented in detail hereunder.

### **Microorganisms and pre-culture 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. Following this protocol, cell cultivation yielded early-stationary phase populations (109 CFU/mL), which were used for further inoculation.

### **Growth stage prior to CAP inactivation**

The growth stage prior to CAP treatment and storage is similar as described in Smet et al. (19). For cells grown in a liquid environment, the amount of salt (0 or 6% (w/v) NaCl (Sigma Aldrich, MO, USA)) was added to TSB without dextrose (Becton, NJ, USA) and if needed the pH (DocuMeter, Sartorius, Goettingen, Germany) was adapted by the addition of 5 M HCl (Acros Organics, NJ, USA). Cells were grown in petri dishes (diameter 5.5 cm) filled with 7 mL of the medium inoculated at 103 CFU/mL, obtained by serial decimal dilutions of early stationary phase cells.

To create a solid(like) environment, gelatin at 5% (w/v) (gelatin from bovine skin, type B, Sigma-Aldrich, MO, USA) together with the appropriate amount of NaCl, was added to TSB. The medium was heated at 60°C in a thermostatic water bath (GR150-S12, Grant Instruments Ltd, Shepreth, UK), the pH adapted, and filter-sterilized using a 0.2 mm filter (Filtertop, 150 mL filter volume, 0.22 µm, TPP, Switzerland). Next, 7 mL was pipetted into sterile petri dishes (diameter 5.5 cm) and left to solidify. Finally, the solidified petri dishes were surface inoculated at approximately 3.0 x 102 CFU/cm2 (surface area 23.8 cm2, corresponding to 103 CFU/mL), by spreading 20 μL of the properly diluted inoculum onto each petri dish. Regardless the growth morphology, petri dishes were sealed with parafilm and placed in a temperature controlled incubator (KB 8182, Termaks, Bergen, Norway) at 20°C under static conditions until the early stationary phase was reached.

### **Sample inoculation for CAP inactivation**

When the early stationary phase was reached, samples were diluted (using dilution medium with the same NaCl concentration and pH value as the growth medium) in order to obtain a cell density of 5.5 log(CFU/mL) (when inactivated in a liquid carrier) or 5.5 log(CFU/cm2) (when inactivated on a solid(like) surface). In case of surface colonies, the sample was melted in a thermostatic water bath (GR150-S12, Grant Instruments Ltd, Shepreth, UK) at 37°C prior to dilution.

Finally, 100 µL of the diluted medium was pipetted on empty 5 cm petri dishes in case inactivation studies were performed in a liquid carrier. Regarding inactivation on a solid(like) surface, the gelled surface was prepared in a 5 cm petri dish (surface area 19.6 cm2) at similar experimental conditions. 50 µL of the diluted sample was pipetted and spread on the gelled surface, which was allowed to dry in the laminar flow cabinet (Telstar Laboratory Equipment, Woerden, the Netherlands).

### **CAP: equipment and inactivation procedure**

A Dielectric Barrier Discharge (DBD) reactor used to study microbial inactivation (Figure 1). An enclosure (22.5 cm x 13.5 cm x 10 cm) around the electrode increased the residence time of the plasma species around the sample and provided a more controlled environment. The residence time in the enclosure, not airtight, was approximately 45 s. The plasma was generated in a gas mixture of helium (purity 99.996%, at a flow rate of 4 L/min) and oxygen (purity ≥ 99.995%, at a flow rate of 40 mL/min), with a total flow rate of 4.04 L/min. Samples were placed between the 0.8 cm gap of the DBD electrodes, and after flushing the reactor with the helium-oxygen gas mixture for 4 min, the plasma was generated. Samples were treated for 5 minutes at a peak-to-peak voltage around 7 kV, frequency of 15 kHz and dissipated plasma power of 9.6 W. The temperature increase of the sample, measured directly after treatment using a thermometer, was about 2°C.

### **Storage procedure and microbiological analysis**

After treatment, petri dishes were immediately sealed with parafilm, and stored in a temperature controlled incubator at either 8 or 20°C. In some cases, the liquid carrier significantly evaporated due to the CAP generated, thus (for all samples of this type) 100 µL of dilution medium at the same experimental conditions was added prior to storage. Control tests, executed without current, confirmed that this evaporation was because of the CAP generation and not due to the gas flow itself (18).

During the storage period (10 days if stored at 20°C, 30 days if stored at 8°C) sampling took place at regular time intervals (depending on the storage temperature). The cell density during this storage period of both CAP treated samples and the controls, was determined by plate counts on both general and selective media (see below). Plate counts on selective media were included in the protocol, to assess sublethal injury (SI).

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 collected from the petri dish and 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. For each sample, 3-6 dilutions were plated in duplicate onto TSA plates (general media) and XLD-Agar (Xylose Lysine Deoxycholate Agar, Merck & Co, New Jersey, USA). The drop technique was used plating by three drops of 20 µL per dilution on TSA and XLD agar plates (21). TSA plates (general media) were placed at 37°C for 24 h before counting, while selective plates (XLD Agar) were stored up to 48 h at 30°C. Cell counts shown in the figures are the mean of all countable dilutions and duplicates for each sample.

### **Modelling and parameter estimation**

If growth was observed, experimental data were fitted with the primary growth model of Baranyi and Roberts (22):

(1)

where *N(t)* [CFU/mL] is the cell density at time *t* [h], *Nmax* [CFU/mL] is the maximum cell density at the stationary phase, *μmax* [h-1] is the maximum growth rate and *Q(t)* [-] is a measure of the physiological state of the cells.

The model of Geeraerd et al. (23), was used to fit experimental data in case inactivation was observed. This model describes a microbial inactivation curve consisting of a shoulder, a loglinear inactivation phase and a tail:

(2)

where *N(t)* [CFU/mL] is the cell density at time *t* [h], *Nres* [CFU/mL] is the residual cell density at the tailing phase, *kmax* [h-1] is the maximum inactivation rate and *Cc(t)* [-] is a measure of the physiological state of the cells.

Parameters of both models were estimated via the minimization of the sum of square errors (SSE), using the *lsqnonlin* routine of the Optimization Toolbox of Matlab version R2009b (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.

## Influence of CAP on model system properties

Additionally, it was investigated if CAP treatment is able to influence the properties of the model systems. In order to study this impact, pH values and aw values (representing the salt concentration) for both CAP treated samples and controls were measured. In order to assess the impact on the food (micro)structure, oscillatory rheological measurements of the gelled support system (CAP treated or controls) were performed. Storage parameters (t=0 days, t=10 days at 20°C or t=30 days at 8°C) were included in these assessments. All experiments were performed in triplicate.

### **Influence of CAP on pH and aw**

Both support systems (the liquid carrier and the solid(like) surface) at the two experimental conditions (pH 7.4, 0% (w/v) NaCl and pH 5.5, 6% (w/v) NaCl) were prepared according to the protocol described in Section 2.1.2. In order to determine pH and aw, the liquid carrier consisted of 5 mL of medium instead of the 100 µL used in Section 2.1. The pH (DocuMeter, Sartorius, Goettingen, Germany) and aw (4TE, Aqualab, Pullman, WA, USA) were determined for both CAP treated samples and controls (storage time t=0 days). CAP treatment follows the protocol described in Section 2.1.4. CAP treated samples and controls were simultaneously incubated and stored for either 10 days at 20°C or 30 days at 8°C. After storage, samples were removed from the incubator and the pH and aw were measured at room temperature.

### **Influence of CAP on microstructural properties: oscillatory rheology of gelled inactivation support systems**

Gelled systems at both pH 7.4, 0% (w/v) NaCl and pH 5.5, 6% (w/v) NaCl were prepared according to the protocol described in Section 2.1.2. Measurements of both CAP treated samples and controls were performed for the three different storage conditions. Regarding CAP treatment, again the same protocol was followed (Section 2.1.4).

Rheological measurements were performed using an AR2000ex stress controlled rheometer (TA instruments, New Castle, USA) equipped with a 40 mm parallel plate-plate system. The plates, gap size 1000 µm, were crosshatched in order to prevent slippage of the sample. The rheometer was supplemented with a Peltier temperature control system and a heated upper plate in order to control the sample temperature. Experiments were conducted at 20°C. Prior to the actual measurement, a delay of 2 minutes was set after sample loading, allowing relaxation of stresses induced during loading. Excess material was wiped off using a spatula.

By running stress sweeps between 1 and 10000 Pa at a fixed frequency of 1 Hz, the linear viscoelastic region (LVR) of the gels was determined. Stress sweeps were conducted until structure breakdown. Dynamic oscillatory frequency sweeps were conducted at a constant oscillation stress of 20 Pa, a stress selected within the LVR (determined during preliminary experiments). The frequency sweep tests were performed in a frequency range of 0.1 to 10 Hz. The storage modulus (G’, a measure of elastic properties) and loss modulus (G”, a measure of viscous properties) were obtained from the software (Rheology Advantage, TA version 5.7).

## Statistical analysis

Analysis of variance (ANOVA) test was performed to determine whether there were significant differences amongst means of logarithmically transformed viable counts and model system property parameters, at a 95.0% confidence level (α = 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.

# RESULTS AND DISCUSSION

The growth and inactivation curves in Figure 2 (20˚C, storage for 10 days) and 3 (8˚C, storage for 30 days) illustrate the influence of CAP treatment (red) as compared to untreated controls (black), under different experimental conditions. The growth or inactivation parameters, obtained by fitting either the growth model (Equation 1) or the inactivation model (Equation 2), are represented in Table 1 and 2, together with statistical analysis.

For the figures and tables, both the total viable population and uninjured viable population are implemented. Cells can become sublethally injured as a result from the treatment, which may pose public health concerns since sublethal injured cells are susceptible to recovery (24). For the selected experimental conditions (after 5 min of CAP treatment), the percentage of SI ranges from 40 up to 90%, indicating cells become sublethally injured due to CAP treatment (19). However, as (statistical) differences between both populations proved to be insignificant during the subsequent storage period (Figure 2 and 3), statistical analysis regarding SI it is not included in the table, neither discussed in the further text.

## Influence of intrinsic factors on the inactivation efficacy of CAP

Evidently, CAP treatment reduces the cell density at the start of the storage period. This results in initial cell densities at the start of storage (*N0*) which are significantly lower as compared to the controls. However, the efficacy of the CAP treatment is affected by the intrinsic factors of the model system.

Smet et al. (19) reported that severe osmotic stress preceding CAP treatment, induced during cell growth, results in cells resistant towards subsequent stresses (25). As indicated by the results in Table 1 and 2, stress resistance towards CAP results in high *N0* values for CAP treated samples, if grown under severe osmotic stress (pH 5.5, 6% (w/v) NaCl). Higher reductions, and thus lower *N0* values, are obtained for cells grown under optimal conditions (pH 7.4, 0% (w/v) NaCl).

When assessing the influence of the growth morphology on *N0*, a specific trend emerges, as surface colonies often have a higher initial cell density than planktonic cells. Like stress resistance against CAP treatment occurs at pH 5.5, 6% (w/v) NaCl, the growth morphology can also induce resistance towards CAP treatment. Nutrient limitations for cells grown as surface colonies lead to starvation stress (26). As indicated by the results, starvation stress promotes resistance towards CAP treatment (18, 19).

As the CAP efficacy (and thus *N0*)is clearly determined by intrinsic factors, this influences as well the relation between CAP treated samples and controls during the storage period. If stress resistance towards CAP treatment arises, it might as well affect the storage life.

## Microbial behavior during storage

### **Influence of CAP on the microbial behavior during storage**

Regardless the experimental growth or storage conditions, CAP treatment extends the storage life. In most cases, this extension is realized by slowing down microbial growth, as it is the case for most experiments at 20°C, or for pH 7.4, 0% (w/v) NaCl at 8°C. In other cases, the cell densities are even further reduced (e.g., pH 5.5, 6% (w/v) NaCl at 8°C). Noteworthy, for this severely stressing condition at a low temperature, also untreated control samples display an inactivation of the cell level. Figure 2 and 3 illustrate the different tendencies during storage, while Figure 4 provides a visual interpretation.

When assessing storage parameters for experimental cases where growth was observed, statistical analysis regarding the lag phase only indicates significant differences at 8°C, where CAP treated samples have significantly longer lag phases as compared to the controls. Differences in maximum specific growth rates between CAP treated samples and the untreated controls depend on the experimental condition. For the most optimal growth condition, pH 7.4, 0% (w/v) NaCl at 20°C, growth patterns for CAP treated samples and controls are much alike, therefore *µmax* values are not significantly different. *µmax* values are alike for this optimal experimental condition at 8°C, as the difference in growth behavior is mainly caused by the extension of the lag phase. If growth during storage resulted in a stationary phase, *Nmax* values are either similar for both CAP treated samples and controls, or lower for treated samples.

When inactivation is observed during storage, a shoulder phase is rarely present, thus no conclusions regarding its length can be drawn. Again, differences in inactivation rates (*kmax*) depend on the experimental condition. At 20˚C and for cells grown at pH 5.5, 6% (w/v) NaCl (inactivation in a liquid carrier) CAP treated cells resume to inactivate during storage. Finally, for all experiments at pH 5.5, 6% (w/v) NaCl and 8°C, both CAP treated samples and controls (further) inactivate during storage. However, differences in *kmax* are again limited. For pH 5.5, 6% (w/v) NaCl at 8°C, the residual cell densities in the tail are the lowest for CAP treated samples. As standard errors, especially for *µmax* or *kmax*, are rather high for some experimental conditions, this adds to the lack in statistical significant differences.

As indicated by the figures and statistical storage parameter analysis, CAP treatment has a positive influence on food storage. In literature, Surowsky et al. (27) illustrated that additional storage of CAP treated apple juice inoculated with *Citrobacter freundii* further reduces the microbial counts. Lacombe et al. (28) studied the influence of CAP treated blueberries on native microbiota, after storage up to 7 days at 4˚C. Cell reduction persists throughout the complete storage period. Similar results are reported by Klockow & Keener (29), for storage of spinach leaves at 5˚C for 24 hours. This further increase in cell level during storage is comparable to findings reported for experiments at pH 5.5, 6% (w/v) NaCl stored at 8°C (or in some cases even when stored at 20°C). Tappi et al. (30) studied the effect of CAP on storage of melons (at 10˚C during 4 days) and observe a delayed growth of spoilage mesophilic and psychrotropic microflora if CAP treated. In this study, a delayed cell growth was observed for the optimal growth condition if stored at 8°C, and for most experimental conditions stored at 20°C.

The beneficial effect of CAP treatment on the elongation of the storage period is very distinct, and proved by both literature and the presented results. In Section 3.1, it is discussed how stressing intrinsic factors can create cells resistant to CAP treatment. The beneficial effect of CAP on the storage life is obvious for all experimental results presented. Therefore, CAP treatment itself (as a stress factor) does not induce cells’ resistant to stressing storage conditions. These results also indicate that microbial growth or inactivation during storage is always affected by both intrinsic and extrinsic factors. The impact of CAP on the microbial kinetics during storage is also affected by the environmental factors, for example the different storage temperatures. Additionally, intrinsic factors of the food (model) system prove to have an effect. The impact of all of these factors on food preservation by CAP treatments are discussed in detail in the following sections.

### **Influence of temperature on the microbial behavior during storage**

Temperature is an important extrinsic factor influencing microbial dynamics. During storage at 20˚C, *S.*Typhimurium cells are almost always able to grow, CAP treated or not. At 8˚C, the low temperature affects the cells ability to recover and grow, possibly even resulting in further inactivation (pH 5.5, 6% (w/v) NaCl).

Whenever growth is observed, the impact of the storage temperature on the growth parameters is evident (Table 1 and 2). Lag phase estimates are extended at 8˚C as compared to results at 20˚C, while both the maximum specific growth rate and the maximum cell density are significantly higher at 20˚C. At suboptimal temperatures, microbial growth is slowed down, resulting in a reduction of growth rates and an elongation of the lag phase. This is due to a decrease of enzymatic reactions within the microbial cell, in combination with changes in its membrane structure (31, 32). In case the cell level further reduces during storage, the impact of the temperature on the parameters is difficult to assess for the results obtained. At 20°C, inactivation is only observed at pH 5.5, 6% (w/v) NaCl for samples treated on a liquid carrier, and statistical differences between parameters of this condition at 8 and 20°C are not significant, so it is not possible to assess the influence of the storage temperature on the inactivation parameters.

When assessing the relation between CAP treated samples and controls at both temperatures, it can be concluded that storage at a lower temperature prolongs the positive effect of CAP treatment on the microbial kinetics. This effect is illustrated at pH 7.4, 0% (w/v) NaCl, by the lag time elongation of CAP treated samples at 8˚C as compared to 20˚C. After storage of 30 days following CAP treatment, cells at 8˚C are not able to reach a stationary phase, while control samples (in most cases) do. At 20˚C, the stationary phase is reached within 48 hours, for both CAP treated samples and controls. The additional effect of temperature on the microbial behavior after CAP treatment is as well notable at pH 5.5, 6% (w/v) NaCl for cells inactivated on a solid(like) surface. At 20°C, CAP treatment reduces growth as compared to the controls, while at 8°C both CAP treated samples and controls are no longer able to survive. Regardless the experimental conditions, the relation in microbial growth behavior between CAP treated samples and untreated controls completely adapts according to the temperature, indicating the additional benefit of a low storage temperature after CAP treatment.

Similar findings regarding the effect of the storage temperature after CAP treatment, in comparison to the controls, have previously been reported by Song et al. (33). In that study, 10 days of storage of CAP treated lettuce results in a further reduction of the *E. coli* cell level when stored at 4˚C, while growth is observed when stored at 10˚C. Inactivation on lettuce (pH 6.0-6.5, aw up to 1.0) can be best compared to our system of surface colonies grown at pH 7.4, 0% (w/v) NaCl, for cells inactivated on a solid(like) surface (h). In this case, regardless the storage at 8 or 20°C, a (reduced) growth is always observed (as both temperatures selected are not as severe as 4°C). Furthermore, in Song et al. (33) the controls follow the same trend as the treated samples at both temperatures tested. This is in contrast with results reported at 20˚C for pH 5.5, 6% (w/v) NaCl (inactivation for CAP treated samples, growth for controls). Differences with findings from literature already indicate the additional influence of other (intrinsic) factors on the storage behavior after CAP treatment.

### **Influence of osmotic stress and suboptimal pH on the microbial behavior during storage**

Next to extrinsic factors, also the intrinsic factors of the food product or model system influence microbial dynamics during storage. In this study, two different levels of composition of the medium are assessed, influencing the storage parameters: (i) pH 7.4, 0% (w/v) NaCl, a condition close to the optimum for growth of *S.*Typhimurium and (ii) a severely stressing condition at pH 5.5, 6% (w/v) NaCl.

Whereas for the optimal experimental condition (slow) growth is always observed for CAP treated samples and untreated controls, increasing the acidic and osmotic stress level slows down growth and can even result in a shift towards microbial inactivation.

As at 8°C, different inactivation types were observed for both experimental conditions, thus it is not possible to compare storage parameters for the experimental conditions, but the effect of a stressing experimental condition becomes abundantly clear. At 20˚C for the storage kinetics expressing growth, the optimal condition (pH 7.4, 0% (w/v) NaCl) introduces shorter lag phases, higher *µmax* values and higher maximum cell densities during the storage period as compared to the condition at pH 5.5 and 6% (w/v) NaCl. Similar results were reported for both planktonic cells and surface colonies in Boons et al. (34) and Smet et al. (35), where the influence of osmotic and acidic stress on the growth morphology of *S.*Typhimurium cells is studied. The addition of the salt in the medium reduces the water activity, exerting severe stresses on the microorganisms and possibly resulting in cell death (36). As in Smet et al. (37), the suboptimal pH values selected in this study are mild and far from growth limits, as *S.*Typhimurium has a *pHmin* of 3.8 and *pHmax* of 9.5 (38). Therefore, the pH influence on the storage behavior is likely to be lower as compared to the impact due to osmotic stress.

As for temperature, also (stressing) media conditions influence the microbial dynamics and are able to amplify the effect of CAP, further extending the food storage period. At 20˚C for pH 5.5, 6% (w/v) NaCl, the increasing stress level differentiates the microbial kinetics of CAP treated samples from controls, generating slower growth (inactivated in a solid(like) carrier) or inactivation (inactivated in a liquid carrier). The shift towards inactivation is also notable at 8˚C, but the additional effect of stressing media conditions in combination with CAP treatment is not as distinct as inactivation was also monitored for the controls.

### **Influence of food structure on the microbial behavior during storage**

A final intrinsic factor taken into account is the influence of the food structure. On the one hand, the food structure may be influenced by the morphology of the cells, i.e., planktonic cells or surface colonies, during the growth phase prior to the CAP treatment. On the other hand, the support system during CAP treatment and storage plays an important role: cells can either be inactivated and stored in a liquid carrier or on a solid(like) support. During further storage, the type of carrier will again determine the cell morphology (planktonic cells or surface colonies).

#### **Influence of the initial growth morphology of the cells.** The influence of the initial growth morphology (prior to CAP treatment) on the storage behavior is limited, as indicated by the figures and the lack of statistically significant differences regarding parameters. The effect of the initial growth morphology is limited to its impact on the CAP efficacy itself (Section 3.1).

#### **Influence of the support system.** Finally, the support system in/on which cells are CAP treated and stored affects the storage behavior. If cells survive and reproduce during storage, it is expected that planktonic cells in the liquid carrier grow faster (shorter lag phase, higher µmax and Nmax values) as compared to surface colonies in the solid(like) support system (39, 40). In other cases studying the influence of the growth morphology, e.g., at suboptimal temperatures or under osmotic stress, growth rates in a liquid and solid(like) system are alike (35, 37). As for the initial growth morphology, also the influence of the support system during storage on the parameters is small. In most cases, no significant differences in the maximum specific growth rates (or inactivation rates) regarding cells in both the liquid and solid(like) support system are detected.

There is one exception to this observed behavior, at 20°C for cells grown at pH 5.5, 6% (w/v) NaCl. Cells CAP treated on a solid(like) surface are able to recover and grow, while cells in a liquid carrier are further inactivated during storage. A first explanation could be that long lived species like H2O2 are produced in the liquid carrier. For low concentrations of H2O2, low pH values enhance its antimicrobial effect (41). This could explain why only at pH 5.5 and 6% (w/v) NaCl, further inactivation during storage was monitored for cells treated in a liquid carrier. Secondly, for this specific experimental condition, it could be stated that the choice of a liquid carrier as a support system enhances the effect of the CAP treatment. However, the two support systems are not comparable, as the liquid carrier exists of only 100 µL of media, a restriction in order to facilitate sufficient log reduction after CAP treatment. The nutrient level available in the liquid carrier is not comparable to the solid(like) surface, and might not be sufficient for microbial recovery and growth of CAP treated cells under certain experimental stressing conditions (pH 5.5, 6% (w/v) NaCl). This fact could contribute to differences observed between the different support systems at 20°C and pH 5.5, 6% (w/v) NaCl.

## Influence of CAP on model system related properties during storage

### **Influence on pH and aw**

Table 5 illustrates the influence of CAP on both intrinsic factors. As indicated by statistical analysis, CAP treatment tends to slightly decrease the pH, as was previously reported by Shi et al. (42) when assessing the influence of CAP on the product properties of orange juice. The storage type (time and temperature), has no specific impact on the pH measurement. However, standard errors on pH data tend to increase when storage time increases, especially at pH 7.4, 0% (w/v) NaCl. It should be noted that samples were more likely to be contaminated at this optimal condition, as compared to the media at pH 5.5, 6% (w/v) NaCl. Contamination of the samples was not (yet) detectable, yet it could explain the higher variation on the data.

Neither CAP treatment, storage time or temperature have a significant influence on the aw of the model systems investigated (Table 5). This corresponds to the findings reported in Song et al. (33), regarding the use of CAP treatment for preservation of fresh lettuce.

### **Influence on microstructural properties: rheology of gelled systems**

The viscoelastic properties of the gelled systems are studied by performing dynamic oscillatory measurements in order to determine structural changes in the gelled systems resulting from CAP treatment.

#### **Oscillatory stress sweep (length of the LVR).** The LVR is defined as the stress range over which G’ and G” are independent of the stress amplitude applied, indicating the structure of the system remains intact. This is an indicator regarding the stability of the system (43). The stress at which the system starts to show nonlinear viscoelastic behavior is defined by the critical shear stress (σc) (44), represented in Table 6. σc is calculated from the stress sweep curves (not shown), as the point where the complex modulus G\* () deviates more than 5% from the previous G\* value. This critical shear stress thus indicates the onset of the nonlinear region where the structure starts to deform under the applied stress (45). When comparing σc values for CAP treated samples and controls, no statistical differences are present, indicating that CAP treatment does not influence the resistance to external stress and the systems stability. The only significant difference can be found at pH 7.4, 0% (w/v) NaCl and 0 days of storage. Also when assessing the influence of the composition of the medium (pH 7.4, 0% (w/v) NaCl vs. pH 5.5, 6% (w/v) NaCl), almost no statistical differences are detected. However, although not significant, the addition of salt in combination with a lower pH resulted in a decrease of σc values. Therefore, it can be concluded that the gel at pH 7.4, 0% (w/v) NaCl is stronger and more stable as compared to the gel at pH 5.5, 6% (w/v) NaCl. Finally, statistical differences are found for the different storage times, but no trend can be concluded.

#### 

#### **Oscillatory frequency sweep.** The influence of the frequency within the LVR on the dynamic moduli G’ and G” is illustrated in Figure 5 and G’ and G” values (at 1 Hz) are presented in Table 6. For all experimental conditions in the frequency range studied, G’ is higher as compared to G”, and is largely independent of the frequency, indicating strong gel behavior (46, 47, 48).When comparing G’ and G” values of CAP treated samples and controls at pH 5.5, 6% (w/v) NaCl at the start of storage (0 days (a)), both moduli are higher for the untreated controls. However, when measured during storage (after 10 (c) or 30 days (e)) this difference in G’ or G” for CAP and controls is absent, as also indicated by the figures. This indicates that the influence of CAP on the rheology of the system is negligible during storage. The same trend can be deducted at pH 7.4, 0% (w/v) NaCl (b, d, f).

Similar to the stress sweep results, the influence of the addition of salt to the medium in combination with a lower pH is again represented in G’ and G” (frequency sweeps, Table 6). Both moduli are significantly lower at pH 5.5, 6% (w/v) NaCl, indicating that the addition of salt induces a weaker gel as compared to the gel at pH 7.4, 0% (w/v) NaCl, as it can also withhold lower stresses before structure deformation occurs (Section 3.3.2.1). The decrease in moduli for samples at pH 5.5, 6% (w/v) NaCl is probably due to the screening-off of short range electrostatic interactions, thus decreasing the ability for α-chains to come into contact and form electrostatic bridges. (49). This prevents strong gel junctions, essential to the establishment of a continuous network of entanglements, to be formed (50). Although studied under different experimental conditions (measurements G’ and G” after 2 h at 4°C), Haug et al. (49) concluded that G’ is expected to remain constant between pH 5 and 7. As for the range studied (pH 5.5-7.4) the pH effect on G’ and G” is probably negligible, the impact of the addition of salt on the rheology of the system is accentuated.

Finally, statistical differences in G’ and G” are found when comparing different storage times, but again no trend can be concluded.

# CONCLUSION

This study indicates that CAP treatment extends the storage life of food products. Intrinsic factors have been previously reported to influence the CAP inactivation efficacy, as stressing intrinsic factors can create cells resistant to CAP. However, the stressing CAP treatment itself does not induce cells resistant to stressing storage conditions, as the beneficial effect of CAP during the storage life is obvious for all experimental conditions. Additionally, the results indicate that the microbial storage behavior is influenced by both intrinsic and extrinsic factors. As expected, a low storage temperature prolongs the positive effect of CAP treatment on the microbial kinetics, as cell recovery proves to be more difficult at low storage temperatures as compared to storage at room temperatures. Next to the storage conditions, the intrinsic factors of the sample itself again play an important role. Stressing media conditions, e.g., osmotic stress or a suboptimal pH value, can also amplify the positive CAP effect further extending the storage life. The food structure (during growth, treatment and storage) of the model system influences the CAP inactivation efficacy, but its impact on the storage life is minimal.

The effect of CAP on the properties of the treated product proved to be minimal. The CAP influence on the intrinsic parameters (pH, aw, food structure) is negligible directly after CAP treatment, and is even further reduced during storage.

This research proves CAP treatment is able to extend the storage time of a food product, depending on the storage conditions and intrinsic parameters of the product. Next to the ability of CAP to obtain high microbial reductions, the additional prolonged food storage enhances the suitability of CAP to be applied in the food industry.

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# FIGURE LEGENDS

**Figure 1.** (a) the CAP set up: (1) plasma power source, (2) Dielectric Barrier Discharge reactor (22.5 cm x 13.5 cm x 10 cm), (3) DC power supply, (4) oscilloscope and (5) function generator. (b) DBD electrode inside reactor (electrode: diameter 5.5 cm, dielectric: 7.5 cm) petri dish containing sample: petri dish with diameter 5 cm). (c) Schematic representation DBD electrode with sample.

**Figure 2.** Growth/ inactivation curves of *S.* Typhimurium, both for CAP treated samples and untreated controls, stored for 10 days at 20°C. Prior to CAP treatment, cells were grown at pH 5.5, 6% (w/v) NaCl (a-d) or pH 7.4, 0% (w/v) NaCl (e-h), as planktonic cells (a, b, e, f) or as surface colonies (c, d, g, h). Cells were inactivated in a liquid carrier (a, c, e, g) or on a solid(like) surface (b, d, f, h). Experimental data (symbols) and global fit (line) of the Baranyi and Roberts (1994) model (growth), or the Geeraerd et al. (2000) model (inactivation): total viable population (o, solid line) and uninjured viable population (x, dashed line).

**Figure 3.** Growth/ inactivation curves of *S.* Typhimurium, both for CAP treated samples and untreated controls, stored for 30 days at 8°C. Prior to CAP treatment, cells were grown at pH 5.5, 6% (w/v) NaCl (a-d) or pH 7.4, 0% (w/v) NaCl (e-h), as planktonic cells (a, b, e, f) or as surface colonies (c, d, g, h). Cells were inactivated in a liquid carrier (a, c, e, g) or on a solid(like) surface (b, d, f, h). Experimental data (symbols) and global fit (line) of the Baranyi and Roberts (1994) model (growth), or the Geeraerd et al. (2000) model (inactivation): total viable population (o, solid line) and uninjured viable population (x, dashed line).

**Figure 4.** CAP treated samples (top) and controls (bottom) for *S.* Typhimurium cells inactivated on a solid(like) surface: a) Planktonic cells grown at pH 7.4, 0% (w/v) NaCl, stored at 20°C for 10 days (images after 1 day and 10 days of storage). b) Planktonic cells grown at pH 5.5, 6% (w/v) NaCl, stored at 20°C for 10 days (images after 4 and 10 days of storage). c) Planktonic cells grown at pH 7.4, 0% (w/v) NaCl, stored at 8°C for 30 days (images after 8 and 30 days of storage).

**Figure 5.** Frequency dependence of storage modulus (G’), loss modulus (G’’) of the gelled inactivation support systems.

# TABLES

Table 1. Growth and inactivation parameters for *S.* Typhimurium stored at 20°C. Cells were inactivated and stored on a liquid carrier or on a solid(like) surface. Cells were grown at pH 5.5, 6% (w/v) NaCl, and either planktonically or as surface colonies. Both CAP treated samples and untreated controls were stored.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Support system  (inactivation + storage)** | **Growth morphology**  **(+ number)** | **Treatment** | **Population** | **Inactivation parameters** | | | | **RMSE** |
| **12 logN0 34 5 (log(CFU/mL))/ 12 logN0 34 5 (log(CFU/cm2))** | **12 tl (h) 34 5** | **12 kmax (1/h) 34 5** | **12 logNres 34 5 (log(CFU/mL)) / 12 logNres 34 5 (log(CFU/cm2))** |
| Liquid carrier | Planktonic cells (a) | CAP treated | Total | 1a 5.1 ± 0.1a2 | 1 85.6 ± 27.21 | 2 0.029 ± 0.0041 | - | 0.2870 |
| Uninjured | 1a 4.8 ± 0.1a1 | 1 54.4± 23.31 | 2 0.027 ± 0.0041 | - | 0.3070 |
| Surface colonies (c) | CAP treated | Total | 1a 4.5 ± 0.2a1 | 1 93.3 ± 117.21 | 1 0.016 ± 0.0111 | - | 0.6999 |
|  | Uninjured | 1a 4.9 ± 0.3a1 | 1 83.9 ± 85.51 | 1 0.022 ± 0.0121 | - | 0.8751 |
|  |  |  | **Growth parameters** | | | | **RMSE** |
|  |  | **12 logN0 34 5 (log(CFU/mL))/ 12 logN0 34 5 (log(CFU/cm2))** | **12 lag (h) 34 5** | **12 µmax(1/h) 34 5** | **12 logNmax 34 5 (log(CFU/mL)) / 12 logNmax 34 5 (log(CFU/cm2))** |  |
| Planktonic cells (a) | Control | Total | 2b 5.8 ± 0.1b2 | b 26.3 ± 0.8B2 | a 0.478 ± 0.101B2 | a 8.1 ± 0.02 | 0.1078 |
| Uninjured | 2b 5.5 ± 0.1b2 | a 25.1 ± 1.4A1 | a 0.441 ± 0.146A2 | a 8.0 ± 0.12 | 0.1849 |
| Surface colonies (c) | Control | Total | 1a 4.9 ± 0.1b1 | a 9.0 ± 5.6A1 | a 0.193 ± 0.062A1 | a 7.9 ± 0.01 | 0.1326 |
| Uninjured | 1a 4.6 ± 0.2a1 | a 0.0 ± 21.2A1 | a 0.111 ± 0.082A1 | a 7.6 ± 0.11 | 0.2956 |
| Solid(like) surface | Planktonic cells (b) | CAP treated | Total | 1b 4.3 ± 0.4a1 | a 66.9 ± 95.0a1 | a 0.125 ± 0.208a1 | a 7.6 ± 0.4a1 | 1.0559 |
| Uninjured | 1b 3.9 ± 0.1a1 | a 66.9 ± 88.0a1 | a 0.102 ± 0.110a1 | a 7.5 ± 0.1a1 | 1.1131 |
| Control | Total | 1a 5.5 ± 0.4b1 | a 0.5 ± 6.2aA1 | a 0.096 ± 0.013aA1 | a 8.1 ± 0.5a1 | 0.1637 |
| Uninjured | 1a 5.0 ± 0.2b1 | b 38.1 ± 13.2aA1 | a 0.446 ± 0.500aA1 | a 8.0 ± 0.2b1 | 0.5336 |
| Surface colonies (d) | CAP treated | Total | 1a 5.2 ± 0.1a2 | b 24.4 ± 9.8a1 | a 0.087 ± 0.019a1 | a 7.6 ± 0.1a1 | 0.2482 |
| Uninjured | 1a 4.9 ± 0.2a2 | b 25.3 ± 4.1a1 | a 0.329 ± 1.575a1 | a 7.4 ± 0.1a1 | 0.4100 |
| Control | Total | 1a 5.4 ± 0.1a1 | a 21.2 ± 2.6aB2 | a 0.522 ± 0.346aA1 | a 8.0 ± 0.0b1 | 0.1704 |
| Uninjured | 1a 5.1 ± 0.1a1 | b 20.7 ± 3.9aA1 | a 0.472 ± 0.416aA1 | a 7.9 ± 0.1b1 | 0.2076 |

1 Effect temperature: for each condition, support system, growth morphology, treatment and population type, storage parameters of the model bearing different superscripts (no numbers in common) are significantly different (P ≤ 0.05)

2Effect condition: for each temperature, support system, growth morphology, treatment and population type, storage parameters of the model bearing different subscripts (no lowercase letters in common) are significantly different (P ≤ 0.05)

3 Effect condition: for each temperature, condition, support system, growth morphology and population type, storage parameters of the model bearing different superscripts (no lowercase letters in common) are significantly different (P ≤ 0.05)

4 Effect support system: for each temperature, condition, growth morphology, treatment and population type, storage parameters of the model bearing different subscripts (no uppercase letters in common) are significantly different (P ≤ 0.05)

5 Effect growth morphology: for each temperature, condition, support system, treatment and population type, storage parameters of the model bearing different subscripts (no numbers in common) are significantly different (P ≤ 0.05)

**Table 2.** Growth and inactivation parameters for *S.* Typhimurium stored at 20°C. Cells were inactivated and stored on a liquid carrier or on a solid(like) surface. Cells were grown at pH 7.4, 0% (w/v) NaCl, and either planktonically or as surface colonies. Both CAP treated samples and untreated controls were stored.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Support system  (inactivation + storage)** | **Growth morphology**  **(+ number)** | **Treatment** | **Population** | **Inactivation parameters** | | | | **RMSE** |
| **12 logN0 34 5 (log(CFU/mL))/ 12 logN0 34 5 (log(CFU/cm2))** | **12 tl (h) 34 5** | **12 kmax (1/h) 34 5** | **12 logNres 34 5 (log(CFU/mL)) / 12 logNres 34 5 (log(CFU/cm2))** |
| Liquid carrier | Planktonic cells (e) | CAP treated | Total | 2a 4.9 ± 0.2a1 | 1 4.7 ± 2.1aA1 | 2 0.545 ± 0.053aA1 | 9.8 ± 0.1a1 | 0.2495 |
| Uninjured | 2a 4.7 ± 0.2a1 | 1 4.1 ± 2.2aA1 | 2 0.532 ± 0.055aA1 | 9.5 ± 0.1a1 | 0.2766 |
| Control | Total | 1a 5.4 ± 0.2b1 | 1a 5.9 ± 2.7aB1 | 2 a 0.582 ± 0.087aB1 | 2b 9.7 ± 0.1a1 | 0.2287 |
| Uninjured | 1a 5.2 ± 0.1b1 | 1a 13.2 ± 26.9aA1 | 2 a 1.061 ± 2.985aA1 | 1b 9.6 ± 0.1a1 | 0.2224 |
| Surface colonies (g) | CAP treated | Total | 1b 5.3 ± 0.2a1 | 1 6.4 ± 11.7aA1 | 2 0.568 ± 0.359aA1 | 2 9.9 ± 0.1b1 | 0.2867 |
| Uninjured | 1a 5.2 ± 0.2a2 | 1 4.2 ± 5.6aA1 | 2 0.512 ± 0.132aA1 | 2 9.7 ± 0.1a1 | 0.2818 |
| Control | Total | 1b 5.4 ± 0.2a1 | 1a 2.4 ± 2.6aA1 | 1a 1.308 ± 39.985aA1 | 2b 9.6 ± 0.1a1 | 0.2570 |
| Uninjured | 1b 5.4 ± 0.2a1 | 1a 2.7 ± 3.6aA1 | 2b 0.446 ± 0.069aA1 | 2b 9.6 ± 0.1a1 | 0.2426 |
| Solid(like) surface | Planktonic cells (f) | CAP treated | Total | 1a 3.0 ± 0.2a1 | 1a 1.3 ± 2.1aA1 | 2b 0.474 ± 0.037bA2 | b 9.1 ± 0.1a1 | 0.3194 |
| Uninjured | 1a 2.9 ± 0.2a1 | 1a 0.9 ± 2.1aA1 | 2b 0.470 ± 0.036bA2 | b 9.0 ± 0.1a1 | 0.3156 |
| Control | Total | 1a 5.4 ± 0.1b1 | 1a 0.6 ± 1.8aA1 | 2b 0.357 ± 0.028aA1 | 2b 9.1 ± 0.0a2 | 0.1847 |
| Uninjured | 1a 5.3 ± 0.1b1 | 1a 0.0 ± 1.7aA1 | 2a 0.355 ± 0.028aA1 | 2b 9.1 ± 0.0a2 | 0.1791 |
| Surface colonies (h) | CAP treated | Total | 2a 5.1 ± 0.1a2 | 1a 3.0 ± 2.1aA1 | 2b 0.338 ± 0.026aA1 | b 9.0 ± 0.1a1 | 0.2244 |
| Uninjured | 2a 5.1 ± 0.1a2 | 1a 5.0 ± 2.4aA1 | 2a 0.355 ± 0.035aA1 | b 9.0 ± 0.1a1 | 0.2445 |
| Control | Total | 1a 5.3 ± 0.1a1 | 1a 5.8 ± 27.0aA1 | 1a 3.335 ± 129.141aA1 | 2b 8.8 ± 0.1a1 | 0.2522 |
| Uninjured | 1a 5.3 ± 0.1a1 | 1a 5.6 ± 7.9aA1 | 1a 2.593 ± 22.295aA1 | 2b 8.8 ± 0.1a1 | 0.2895 |

1 Effect temperature: for each condition, support system, growth morphology, treatment and population type, storage parameters of the model bearing different superscripts (no numbers in common) are significantly different (P ≤ 0.05)

2Effect condition: for each temperature, support system, growth morphology, treatment and population type, storage parameters of the model bearing different subscripts (no lowercase letters in common) are significantly different (P ≤ 0.05)

3 Effect condition: for each temperature, condition, support system, growth morphology and population type, storage parameters of the model bearing different superscripts (no lowercase letters in common) are significantly different (P ≤ 0.05)

4 Effect support system: for each temperature, condition, growth morphology, treatment and population type, storage parameters of the model bearing different subscripts (no uppercase letters in common) are significantly different (P ≤ 0.05)

5 Effect growth morphology: for each temperature, condition, support system, treatment and population type, storage parameters of the model bearing different subscripts (no numbers in common) are significantly different (P ≤ 0.05)

Table 3. Growth and inactivation parameters for *S.* Typhimurium stored at 8°C. Cells were inactivated and stored on a liquid carrier or on a solid(like) surface. Cells were grown at pH 5.5, 6% (w/v) NaCl, and either planktonically or as surface colonies. Both CAP treated samples and untreated controls were stored.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Support system (inactivation + storage)** | **Growth morphology**  **(+ number)** | **Treatment** | **Population** | **Inactivation parameters** | | | | **RMSE** |
| **12 logN0 34 5 (log(CFU/mL))/ 12 logN0 34 5 (log(CFU/cm2))** | **12 tl (h) 34 5** | **12 kmax (1/h) 34 5** | **12 logNres 34 5 (log(CFU/mL)) / 12 logNres 34 5 (log(CFU/cm2))** |
| Liquid carrier | Planktonic cells (a) | CAP treated | Total | 1b 5.1 ± 0.1a1 | 1 169.1 ± 95.71 | 1 0.006 ± 0.001bA1 | - | 0.1887 |
| Uninjured | 1b 4.8 ± 0.2a1 | 1 0.0 ± 124.11 | 1 0.007 ± 0.001aA1 | - | 0.3152 |
| Control | Total | 1a 5.0 ± 0.1a1 | - | 0.004 ± 0.000aA1 | - | 0.2389 |
| Uninjured | 1a 4.6 ± 0.1a1 | - | 0.007 ± 0.001aA1 | - | 0.4055 |
| Surface colonies (c) | CAP treated | Total | 2a 5.5 ± 0.2a2 | 1 87.9 ± 48.0a1 | 1 0.020 ± 0.004bB2 | 2.3 ± 0.1a | 0.3693 |
| Uninjured | 1a 5.2 ± 0.2a1 | 1 0.0 ± 57.4a1 | 1 0.019 ± 0.004aA2 | 2.2 ± 0.1a | 0.3289 |
| Control | Total | 2b 6.0 ± 0.2b2 | 42.4 ± 92.1a | 0.011 ± 0.003aA2 | 3.8 ± 0.2b | 0.2896 |
| Uninjured | 2a 5.6 ± 0.2a2 | 0.0 ± 104.0a | 0.019 ± 0.003aA2 | 3.2 ± 0.2b | 0.3445 |
| Solid(like) surface | Planktonic cells (b) | CAP treated | Total | 1b 4.0 ± 0.4a1 | - | 0.022 ± 0.011aA1 | 2.2 ± 0.2a | 0.8308 |
| Uninjured | 1b 4.0 ± 0.7a1 | - | 0.371 ± 0.467aA1 | 2.0 ± 0.2a1 | 0.9769 |
| Control | Total | 1a 5.0 ± 0.2b1 | - | 0.012 ± 0.005aA1 | 3.5 ± 0.2b | 0.5312 |
| Uninjured | 1a 4.8 ± 0.2a1 | - | 0.025 ± 0.007aB1 | 2.5 ± 0.2b1 | 0.5083 |
| Surface colonies (d) | CAP treated | Total | 2b 5.5 ± 0.1a2 | - | 0.005 ± 0.002aA1 | - | 0.3738 |
| Uninjured | 2b 5.4 ± 0.2a2 | - | 0.026 ± 0.008aA1 | 3.5 ± 0.2b2 | 0.5120 |
| Control | Total | 1a 5.6 ± 0.1a2 | - | 0.007 ± 0.001aA1 | - | 0.3123 |
| Uninjured | 1a 5.6 ± 0.3a2 | - | 0.029 ± 0.006aA1 | 2.9 ± 0.2a1 | 0.5482 |

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3 Effect condition: for each temperature, condition, support system, growth morphology and population type, storage parameters of the model bearing different superscripts (no lowercase letters in common) are significantly different (P ≤ 0.05)

4 Effect support system: for each temperature, condition, growth morphology, treatment and population type, storage parameters of the model bearing different subscripts (no uppercase letters in common) are significantly different (P ≤ 0.05)

5 Effect growth morphology: for each temperature, condition, support system, treatment and population type, storage parameters of the model bearing different subscripts (no numbers in common) are significantly different (P ≤ 0.05)

Table 4. Growth and inactivation parameters for *S.* Typhimurium stored at 8°C. Cells were inactivated and stored on a liquid carrier or on a solid(like) surface. Cells were grown at pH 7.4, 0% (w/v) NaCl, and either planktonically or as surface colonies. Both CAP treated samples and untreated controls were stored.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Support system (inactivation + storage)** | **Growth morphology**  **(+ number)** | **Treatment** | **Population** | **Growth parameters** | | | | **RMSE** |
| **12 logN0 34 5 (log(CFU/mL))/ 12 logN0 34 5 (log(CFU/cm2))** | **12 lag (h) 34 5** | **12 µmax(1/h) 34 5** | **12 logNmax 34 5 (log(CFU/mL)) / 12 logNmax 34 5 (log(CFU/cm2))** |
| Liquid carrier | Planktonic cells (e) | CAP treated | Total | 1a 4.0 ± 0.3a1 | 2 582.7 ± 92.9bA2 | 1 0.033 ± 0.022aA1 | - | 1.3576 |
| Uninjured | 1a 3.9 ± 0.3a1 | 2 581.2 ± 88.5bA2 | 1 0.034 ± 0.021aA1 | - | 1.3486 |
| Control | Total | 1b 5.5 ± 0.1b1 | 1 0.0 ± 63.6aA1 | 1 0.010 ± 0.001aA1 | 1 8.7 ± 0.32 | 0.2216 |
| Uninjured | 2b 5.6 ± 0.1b1 | 1 0.0 ± 55.1aA1 | 1 0.009 ± 0.001aA1 | 1 10.0 ± 6.01 | 0.1699 |
| Surface colonies (g) | CAP treated | Total | 1a5.3 ± 0.1a2 | 1 0.0 ± 83.9aA1 | 1 0.009 ± 0.002aA1 | 1 8.1 ± 0.4a | 0.2587 |
| Uninjured | 1a 5.3 ± 0.1a2 | 1 0.0 ± 88.7aA1 | 1 0.009 ± 0.002aA1 | 1 8.3 ± 0.6a | 0.2708 |
| Control | Total | 1a 5.5 ± 0.1a1 | 1 0.0 ± 58.7aA1 | 1 0.017 ± 0.004bA2 | 1 7.8 ± 0.1a1 | 0.2603 |
| Uninjured | 1a 5.5 ± 0.1a1 | 1 0.0 ± 71.6aA1 | 1 0.015 ± 0.004aA1 | 1 7.7 ± 0.1a1 | 0.2851 |
| Solid(like) surface | Planktonic cells (f) | CAP treated | Total | 1a 2.6 ± 0.3a1 | 2 586.3 ± 142.9bA1 | 1 0.018 ±0.015aA1 | - | 0.9897 |
| Uninjured | 1a 2.6 ± 0.3a1 | 2 564.0 ± 147.8bA1 | 1 0.017 ± 0.022aA1 | - | 0.9934 |
| Control | Total | 1a 5.3 ± 0.3b1 | 1 0.0 ± 139.4aA1 | 1 0.022 ± 0.020aA1 | 1 7.0 ± 0.11 | 0.4865 |
| Uninjured | 1a 5.2 ± 0.3b1 | 1 0.0 ± 148.3aA1 | 1 0.022 ± 0.022aA1 | 1 6.9 ± 0.21 | 0.5280 |
| Surface colonies (h) | CAP treated | Total | 1a 4.0 ± 0.2a2 | 2 654.3 ± 148.2bB1 | 1 0.017 ± 0.016aA1 | - | 0.7153 |
| Uninjured | 1a 3.7 ± 0.2a2 | 2 597.1 ± 128.7bB1 | 1 0.015 ± 0.010aA1 | - | 0.6839 |
| Control | Total | 1a 5.3 ± 0.3b1 | 1 0.0 ± 204.2aA1 | 1 0.010 ± 0.006aA1 | 1 7.3 ± 0.31 | 0.5073 |
| Uninjured | 1a 5.1 ± 0.3b1 | 1 0.0 ± 181.8aA1 | 1 0.012 ± 0.008aA1 | 1 7.2 ± 0.31 | 0.5311 |

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2Effect condition: for each temperature, support system, growth morphology, treatment and population type, storage parameters of the model bearing different subscripts (no lowercase letters in common) are significantly different (P ≤ 0.05)

3 Effect condition: for each temperature, condition, support system, growth morphology and population type, storage parameters of the model bearing different superscripts (no lowercase letters in common) are significantly different (P ≤ 0.05)

4 Effect support system: for each temperature, condition, growth morphology, treatment and population type, storage parameters of the model bearing different subscripts (no uppercase letters in common) are significantly different (P ≤ 0.05)

5 Effect growth morphology: for each temperature, condition, support system, treatment and population type, storage parameters of the model bearing different subscripts (no numbers in common) are significantly different (P ≤ 0.05)

Table 5. Effect of CAP on pH and aw: for both support systems, at two experimental conditions (pH 5.5, 6% (w/v) NaCl or pH 7.4, 0% (w/v) NaCl), and three different storage protocols (t=0 days, t=10 days at 20°C or t=30d at 8°C).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Support system (inactivation + storage)** | **Condition** | **Storage** | **Treatment** | **1pH (-)23** | **1aw (-)23** |
| Liquid carrier | pH 5.5, 6% (w/v) NaCl | t=0 days | CAP | A5.751 ± 0.026a1 | A0.9627 ± 0.0008a2 |
| Control | A5.746 ± 0.017a1 | A0.9617 ± 0.0011a2 |
| t=10 days, 20°C | CAP | A5.747 ± 0.080a1 | A0.9599 ± 0.0007a1 |
| Control | A5.790 ± 0.005a1 | A0.9601 ± 0.0006a1 |
| t=30 days, 8°C | CAP | A5.784 ± 0.001a1 | A0.9597 ± 0.0008a1 |
| Control | A5.661 ± 0.115a1 | A0.9595 ± 0.0004a1 |
| pH 7.4, 0% (w/v) NaCl | t=0 days | CAP | B7.161 ± 0.011a1 | B0.9965 ± 0.0011a3 |
| Control | B7.195 ± 0.011b1 | B0.9951 ± 0.0017a1 |
| t=10 days, 20°C | CAP | B6.990 ± 0.190a1 | B0.9903 ± 0.0014a1 |
| Control | B7.218 ± 0.058a1 | B0.9928 ± 0.0009a1 |
| t=30 days, 8°C | CAP | B7.093 ± 0.081a1 | B0.9928 ± 0.0003a2 |
| Control | B7.215 ± 0.005a1 | B0.9950 ± 0.0017a1 |
| Solid(like) surface | pH 5.5, 6% (w/v) NaCl | t=0 days | CAP | A5.574 ± 0.007a1 | A0.9581 ± 0.0018a1 |
| Control | A5.635 ± 0.007b2 | A0.9591 ± 0.0054a1 |
| t=10 days, 20°C | CAP | A5.587 ± 0.004a1 | A0.9562 ± 0.0024a1 |
| Control | A5.608 ± 0.002b1 | A0.9574 ± 0.0005a1 |
| t=30 days, 8°C | CAP | A5.583 ± 0.010a1 | A0.9592 ± 0.0020a1 |
| Control | A5.593 ± 0.011a1 | A0.9566 ± 0.0010a1 |
| pH 7.4, 0% (w/v) NaCl | t=0 days | CAP | B6.637 ± 0.013a1 | A0.9800 ± 0.0016a1 |
| Control | B6.745 ± 0.031b2 | A0.9868 ± 0.0012b1 |
| t=10 days, 20°C | CAP | B6.631 ± 0.003a1 | A0.9913 ± 0.0015a3 |
| Control | B6.654 ± 0.001b1 | A0.9925 ± 0.0016a2 |
| t=30 days, 8°C | CAP | B6.658 ± 0.003a1 | A0.9860 ± 0.0031a2 |
| Control | B6.636 ± 0.026a1 | A0.9898 ± 0.0026a12 |

1 Effect condition: for each support system, storage type and treatment type quality parameters bearing different subscripts (no uppercase letters in common) are significantly different (P ≤ 0.05)

2 Effect treatment: for each support system, condition and storage type, quality parameters bearing different superscripts (no lowercase letters in common) are significantly different (P ≤ 0.05)

3 Effect storage: for each support system, condition and treatment type, quality parameters bearing different subscripts (no numbers in common) are significantly different (P ≤ 0.05)

Table 6. Effect of CAP on σc (critical stress), G’ (1 Hz) and G” (1 Hz) of the gelled systems (solid(like) surface): for two experimental conditions (pH 5.5, 6% (w/v) NaCl or pH 7.4, 0% (w/v) NaCl) and three different storage protocols (t=0 days, t=10 days at 20°C or t=30d at 8°C). Rheological measurements are conducted at 20°C.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Condition** | **Storage** | **Treatment** | **Stress sweep data** | **Frequency sweep data** | |
| **1σc (Pa)23** | **1G’ (Pa, 1 Hz)23** | **1G’’ (Pa, 1 Hz)23** |
| pH 5.5, 6% (w/v) NaCl | t=0 days | CAP | A846.97 ± 74.88a12 | A224.13 ± 28.56a1 | A10.83 ± 0.86a1 |
| Control | A786.17 ± 134.12a1 | A447.80 ± 49.04b3 | A13.55 ± 0.38b2 |
| t=10 days, 20°C | CAP | A1082.84 ± 107.42a2 | A181.03 ± 2.66a1 | A19.03 ± 0.46a3 |
| Control | A1093.79 ± 210.95a1 | A186.83 ± 16.62a1 | A18.36 ± 0.46a3 |
| t=30 days, 8°C | CAP | A808.65 ± 180.14a1 | A391.87 ± 61.12a2 | A16.05 ± 2.11b2 |
| Control | A717.53 ± 212.99a1 | A357.53 ± 58.08a2 | A10.98 ± 1.62a1 |
| pH 7.4, 0% (w/v) NaCl | t=0 days | CAP | B1261.62 ± 92.72b1 | B578.07 ± 36.00a2 | B17.18 ± 0.61a1 |
| Control | A846.38 ± 120.81a1 | B761.03 ± 47.86b2 | B20.31 ± 2.20a1 |
| t=10 days, 20°C | CAP | A1025.55 ± 260.78a1 | B434.93 ± 23.67b1 | B33.31 ± 2.52a3 |
| Control | A1306.15 ± 213.01a2 | B393.00 ± 10.20a1 | B29.95 ± 0.34a3 |
| t=30 days, 8°C | CAP | A976.35 ± 404.06a1 | B787.23 ± 31.02a3 | B24.46 ± 1.84a2 |
| Control | A1006.90 ± 310.17a12 | B885.77 ± 47.67b3 | B26.32 ± 0.99a2 |

1 Effect condition: for each storage and treatment type rheology parameters bearing different subscripts (no uppercase letters in common) are significantly different (P ≤ 0.05)

2 Effect treatment: for each condition and storage type, rheology parameters bearing different superscripts (no lowercase letters in common) are significantly different (P ≤ 0.05)

3 Effect storage: for each condition and treatment type, rheology parameters bearing different subscripts (no numbers in common) are significantly different (P ≤ 0.05)