



# Inactivation of *Salmonella* Typhimurium in fresh-cut lettuce during chlorine washing: Assessing the impacts of free chlorine concentrations and exposure times

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## ABSTRACT

The aim of this study was to evaluate, quantify and model the inactivation of *Salmonella* in fresh-cut lettuce during washings with chlorinated water at different free chlorine concentrations (FCC, 0–150 mg/L). Individual fresh-cut lettuce samples (4 cm<sup>2</sup>) were inoculated with a *Salmonella* culture (ca. 4 log CFU/cm<sup>2</sup>) and washed with 100-mL solutions with different FCC for different times (0–150 s). The surviving *Salmonella* cells recovered from samples were enumerated by plate count methodology. A fast decay on *Salmonella* counts was marked in the first 20 s of washing, followed by a slowing down on reductions. A maximum of 2.6 log-decrease was observed after 2.5-min washing regardless of FCC. The log-linear with tail primary model coupled with a linear secondary model was fitted to inactivation data obtained at FCC from 50 to 150 mg/L through global regression analysis, yielding a suitable model to describe *Salmonella* concentrations as a function of FCC and washing times (RMSE = 0.34, R<sub>adj</sub><sup>2</sup> = 0.84). Simulations using the developed model showed inactivation rates varying from 0.17 log CFU/s at 50 mg/L to 0.86 log CFU/s at 150 mg/L. Disinfection models on lettuce are valuable tools for the validation of control measures in the fresh-cut produce industry and for quantitative risk assessments.

## 1. Introduction

Leafy greens are considered important elements of a healthy diet and provide nutrients that protect the human body against chronic diseases, such as cancers, cardiovascular diseases, and metabolic disorders (Stratton et al., 2021). These products are usually subjected to processing operations, including cutting and shredding, being available to consumers in their most convenient format: as ready-to-eat foods. In spite of being healthy and convenient, these foods are considered important vehicles of microorganisms frequently linked to foodborne outbreaks (Callejón et al., 2015; Carstens et al., 2019; Coulombe et al., 2020). Contamination of fresh-cut leafy greens with outbreak causing pathogens can occur at all steps from pre-harvest to consumption (Gil et al., 2015). Cross-contamination has been considered a primary cause of their contamination at processing environments, being the washing operation a critical step affecting microbial levels in these products (Possas & Pérez-Rodríguez, 2023; Smolinski et al., 2018).

*Salmonella* spp. stands out as one of the main causing agents in fresh produce associated outbreaks (Elias et al., 2019). A systematic review and meta-analysis on the prevalence of *Salmonella* spp. in lettuce revealed mean values of 0.028 (95% CI: 0.014–0.042) and 0.064 (95% CI: 0.041–0.087) in developed and developing countries, respectively (Elias et al., 2019). A multistate outbreak resulting in 31 illnesses and 4 hospitalizations in the United States was reported in 2021 after the consumption of packed salads contaminated with *Salmonella* Typhimurium (CDC, 2021). In Europe, several alert notifications concerning the presence of *Salmonella* spp. in leafy greens sold at different countries have been registered by the Rapid Alert System for Food and Feed in the last years (RASFF Portal, References: 2020.3586, 2020.2245).

The use of eco-friendly sanitizing solutions to control cross-contamination of leafy greens, such as plant extracts and essential oils (Arellano et al., 2021; Pablos et al., 2022), organic acids (Lippman et al., 2020) and electrolyzed water (Afar & Hung, 2018; Han et al., 2021) has been extensively investigated in the last years. However, chlorinated

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solutions, mainly based on sodium hypochlorite, remain as the most common disinfection strategy adopted by the fresh produce industry, due to their relatively low price, ease of application and antimicrobial potential (Pablos et al., 2022). Different factors can affect the disinfection efficiency of sodium hypochlorite including the free chlorine level, the pH, the organic matter load in water or produce surface, temperature of water during disinfection, pathogen attachment in produce and the physiological state of microbial cells (Banach et al., 2017; Chen & Hung, 2017; Davidson et al., 2014; López-Gálvez et al., 2019).

Mathematical models have been applied to set a specific relationship between the washing step parameters and the microbial contamination in leafy greens (Abnavi et al., 2019; Pérez Rodríguez et al., 2011). Munther et al. (2015) built a mathematical model of the chlorine decay during the washing process as well as the dynamics of *Escherichia coli* O157:H7 cross-contamination from spinach to lettuce. More recently, Cuggino et al. (2020) developed a polynomial model relating the *Salmonella* Typhimurium inactivation in fresh-cut lettuce during chlorine washing, as a function of free chlorine concentration (0–200 mg/L), washing time (0–110 s), size of lettuce pieces (9–21 cm<sup>2</sup>) and benzyl-isothiocyanate concentration (0–80 mg/L). Additionally, Cuggino et al. (2023) also modelled *Salmonella* growth kinetics in fresh-cut lettuce during storage at 9, 13 and 18 °C following chlorine washing. These mathematical models play an important role on the decision-making process and in the validation of chlorine washing as a control measure in the fresh produce industry.

The objective of this study was to evaluate, quantify and model the inactivation of *Salmonella* Typhimurium in fresh-cut lettuce during disinfection with chlorinated water at free chlorine concentrations ranging from 0 to 150 mg/L, for 0–150 s.

## 2. Material & methods

### 2.1. Bacterial strains and inoculum preparation

The *Salmonella enterica* serovar Typhimurium SL1344 pGT-kan MB185 (LFMFP 690) strain, labelled with green fluorescent protein gene (GFP) and with a gentamicin resistance gene, was used in the experiments. This strain was chosen as it could be differentiated from the autochthonous microbiota of lettuce and had been already used in fate studies with fresh produce (Cuggino et al., 2020; Pablos et al., 2022; Possas, Posada-Izquierdo, et al., 2021). Moreover, this strain exhibited a comparable survival capacity to other *Salmonella* strains in a previous study with fresh produce (Possas, Posada-Izquierdo, et al., 2021). Stock cultures were maintained by regular subculture on Plate Count Agar (PCA, Oxoid, UK) and stored at 4 °C. One day before each experiment, a loopful of a stock culture was transferred to an individual tube containing 5 mL of Tryptone Soy Broth (TSB, Oxoid, UK) and incubated at 37 °C under static conditions until cells reached the early stationary phase (approximately 20 h). After incubation, the TSB was removed by 3 consecutive washing steps with centrifugation (4100 rpm/10 min). At each step, the cell pellet was resuspended in 5 mL of phosphate buffer solution (PBS, pH 7.3 ± 0.2 at 25 °C, Oxoid, UK).

To determine the initial concentration of the cultures, aliquots were serially diluted in 0.1% peptone water (Oxoid, UK) and surface plated onto PCA plates previously supplemented with gentamicin at 15 µg/mL, and fluorescent colonies were counted after incubation at 37 °C for 24 h. A portable UV light (Spectroline® E-series UV lamp, Merck, Germany) aided at quantifying the characteristic colonies, which showed fluorescence when irradiated. The initial concentration of the cultures was nearly 10<sup>9</sup> CFU/mL.

### 2.2. Sample preparation and inoculation

Unprocessed heads of iceberg lettuce (*Lactuca sativa* L.) with similar shape and size (approximately 450 g) were obtained from a local supermarket of Córdoba (Spain) and stored in the laboratory at 4 °C for a

maximum of 2 h before processing. Outer leaves and core were manually removed and discarded. The internal leaves were cut into pieces of 1 × 4 cm (4 cm<sup>2</sup>) with the aid of sterile scalpels and plastic moulds. The plastic models were previously disinfected with 70% ethanol and exposed to UV light for 30 min prior to experiments. The lettuce pieces were then placed inside Petri dishes. Lettuce processing was performed at 4.5 ± 0.5 °C and 70% relative humidity (RH).

With the aid of a micropipette, aliquots of 0.1 mL of the *Salmonella* culture at a concentration of 5 × 10<sup>6</sup> CFU/mL were evenly distributed on lettuce pieces. Once inoculated the lettuce pieces were maintained inside the flow cabinet for 45 min prior to disinfection treatments, to allow lettuce drying and attachment of microbial cells (Cuggino et al., 2020). *Salmonella* concentrations detected in fresh-cut lettuce pieces after this drying period was approximately 10<sup>4</sup> CFU/cm<sup>2</sup>. The drop in concentrations during this period may be associated with the drying stress suffered by *Salmonella* cells.

### 2.3. Chlorine solutions preparation

Solutions with different free chlorine concentrations (FCC), i.e., 0, 25, 50, 75, 100, 150 mg/L, were prepared by diluting a commercial solution of sodium hypochlorite at 10% (Sigma-Aldrich, USA) with sterile distilled water (DW) at 8 ± 2 °C. The FCC range was selected based on previous surveys performed in fresh-cut lettuce processing industries, in which chlorine washing concentrations ranging between 0 and 200 mg/L were reported (data not shown).

The pH of chlorine solutions was adjusted to 6.0–6.5 using hydrochloric acid (Sigma-Aldrich, Spain) to improve the disinfection efficacy of chlorine and simulate real conditions of the fresh produce industry. Aliquots of 100 mL of the washing solutions were dispensed in sterile plastic containers to be used for the disinfection treatments. The FCC was measured prior and after the experiments by using the Multiparameter Bench Photometer HI83214 (Hanna Instruments, Spain). The pH, total dissolved solids (TDS, mg/L), conductivity (µS/cm), salinity (g/L) of the washing solutions were measured using a Multiparameter PCSTestr 3.5 (Oakton, USA). Finally, the chemical oxygen demand (COD) of washing solutions was measured with the thermoreactor HI839800 COD Test Tube Heater (Hanna Instruments, Spain).

### 2.4. Disinfection treatments

Disinfection treatments were carried out at a laboratory scale mimicking fresh-cut lettuce processing at industrial level. For the disinfection treatments, each piece of inoculated lettuce was placed inside an individual container with 100 mL of chlorine solution with different FCC (0–150 mg/L) with the aid of sterile tweezers. Different washing times were evaluated: 0, 5, 10, 20, 30, 60 and 150 s. Although unrealistic in industrial environments, this maximum exposure time (i.e., 150 s) was used to evaluate the effectiveness of longer treatments on the remaining *Salmonella* populations.

After washing, each lettuce piece was placed into an individual tube containing 10 mL of peptone water (0.1%) and sodium thiosulfate (0.1%) (Panreac, Spain), to neutralize the free chlorine and halt the disinfection process. Three replicates per FCC-time combination were carried out. Treatments with DW served as a control. All disinfection experiments were conducted at 4.5 ± 0.5 °C and 70% RH inside a refrigerating chamber to avoid free chlorine losses and to mimic the conditions of the Spanish fresh-produce industry. The experiments were performed in three different days, using different batches of lettuce.

### 2.5. Microbiological analysis

To estimate the number of viable *Salmonella* cells, peptone water tubes containing lettuce pieces were vortex for 2 min and serial tenfold dilutions were performed. Aliquots were surface plated on PCA agar plates previously supplemented with gentamicin at 15 µg/mL and

incubated at 37 °C for 24 h. At the last four sampling times (20, 30, 60, 150 s) pour plating was also performed to reduce the cells' detection limit. Given that a maximum of 1 mL was taken from the 10 mL-containing tubes used to recover cells from lettuce, effective pathogen detection/quantification could only be achieved in tubes containing levels equal to or higher than 10 CFU per tube (i.e., 1 CFU/mL). On this basis, the detection/quantification limit was 10 CFU in 4 cm<sup>2</sup> (i.e., 2.5 CFU/cm<sup>2</sup> or 0.4 log CFU/cm<sup>2</sup>). After incubation, colonies grown in PCA showing fluorescence were counted.

## 2.6. Statistical analysis and data modelling

Microbial concentration on lettuce was expressed per cm<sup>2</sup> and log transformed. Data analysis and modelling were performed using R (R Core Team, 2020). Reductions at different time intervals were computed as the difference between the count observed at time 0 ( $N_0$ , log CFU/cm<sup>2</sup>) and the count observed at different washing times ( $N$ , log CFU/cm<sup>2</sup>), i.e.,  $\log N_0/N$ , and were statistically compared by ANOVA. The statistically different groups were identified by applying the Tukey's honest significant difference (HSD) test. Survival curves were built by plotting the *Salmonella* concentration (log CFU/cm<sup>2</sup>) versus washing times (s).

The one-step global regression analysis was carried out to fit the inactivation model proposed by Geeraerd et al. (2000) (Equation (1)) to the observed data by applying the nonlinear least square (nls) function, using the Gauss-Newton algorithm in R.

$$\log_{10} N = (\log_{10} N_0 - \log_{10} N_{res}) * \exp(-k_{max} * t) + \log_{10} N_{res} \quad \text{Equation 1}$$

where  $\log_{10} N$  is the bacterial concentration (log CFU/cm<sup>2</sup>) at a specific washing time ( $t$ , s);  $\log_{10} N_0$  is the initial bacterial concentration (log CFU/cm<sup>2</sup>);  $k_{max}$  is the inactivation rate (log CFU/s) and  $\log_{10} N_{res}$  is the residual bacterial concentration (log CFU/cm<sup>2</sup>).

Equation (1) was coupled with an empirical model (Equation (2)) to describe the relationship between the inactivation rate  $k_{max}$  and the FCC.

$$k_{max} = a * FCC + b \quad \text{Equation 2}$$

where FCC is the free chlorine concentration of the washing solution (mg/L) and  $a$  (log CFU/(s.mg/L)) and  $b$  (log CFU/s) are regression coefficients.

The 95% confidence interval of  $k_{max}$  prediction as a function of FCC was estimated based on regression coefficients through forward uncertainty propagation using Monte Carlo simulations (Garre et al., 2017). The Root Mean Square Error (RMSE) and the adjusted R<sup>2</sup> ( $R_{adj}^2$ ) were determined as measures of the goodness-of-fit of the models.

## 3. Results and discussion

### 3.1. *Salmonella* reductions on fresh-cut lettuce due to chlorine treatments

The physicochemical parameters of the chlorinated solutions used for fresh-cut lettuce decontamination are shown in Table 1. No statistical differences were found between the pH values of the chlorinated solutions with different FCC ( $p > 0.05$ ), which are within the range of values reported by different authors for simulated wash water, i.e., 6.0–6.5 (Huang et al., 2020; Nou et al., 2011; Pablos et al., 2022). On the other hand, conductivity, TDS, and salinity increased linearly by increasing FCC in wash water from 50 to 150 mg/L, while no significant differences were detected between conductivity and TDS values for chlorine solutions at 0–50 mg/L (Table 1). Greater mean COD values were also noted when increasing FCC and washing times. For instance, at 50 mg/L values increased from 75.67 ± 14.74 to 96.33 ± 55.59 mg O<sub>2</sub>/L during 10 and 150-s washings, respectively. In addition, COD values at 100 mg/L, varied from 257 ± 64.65 to 285 ± 13 mg O<sub>2</sub>/L, at 10 and 150-s washings. The increase in COD values, which is a parameter assessed as measure of chlorine demand, indicate the increase in organic matter in

**Table 1**

Physicochemical characteristics of the chlorinated washing solutions used for fresh-cut lettuce decontamination, prior to experiments.

Physicochemical parameters	Free chlorine concentration (mg/L)					
	0	25	50	75	100	150
Conductivity (µS/cm)	222 ± 39 <sup>a</sup>	184 ± 39 <sup>a</sup>	224 ± 55 <sup>a</sup>	374 ± 31 <sup>b</sup>	536 ± 52 <sup>c</sup>	741 ± 83 <sup>d</sup>
Total dissolved solids (mg/L)	158 ± 28 <sup>a</sup>	132 ± 29 <sup>a</sup>	158 ± 39 <sup>a</sup>	264 ± 18 <sup>b</sup>	384 ± 41 <sup>c</sup>	534 ± 57 <sup>d</sup>
pH	6.08 ± 0.23 <sup>a</sup>	6.18 ± 0.16 <sup>a</sup>	6.08 ± 0.19 <sup>a</sup>	6.13 ± 0.02 <sup>a</sup>	6.14 ± 0.20 <sup>a</sup>	6.17 ± 0.14 <sup>a</sup>
Salinity (g/L)	0.09 ± 0.00 <sup>a</sup>	0.81 ± 0.07 <sup>b</sup>	1.09 ± 0.32 <sup>b</sup>	1.92 ± 0.13 <sup>c</sup>	2.90 ± 0.35 <sup>d</sup>	3.12 ± 0.30 <sup>d</sup>

Mean values ± standard deviations are reported (n = 3).

Same superscript letters in the same row represent the homogeneous groups according to Tukey's multiple comparison of means (95% family-wise confidence level).

wash water (Weng et al., 2016).

*Salmonella* reductions during the disinfection treatments of fresh-cut lettuce are shown in Table 2. The mean concentration of *Salmonella* in inoculated samples before washing (i.e., time 0) was 3.55 ± 0.12 log CFU/cm<sup>2</sup>. No significant reductions were observed during treatments of lettuce with DW (control) or with an FCC solution at 25 mg/L at all washing times evaluated (reductions <0.5 log CFU/cm<sup>2</sup>). Regarding treatments with FCC from 50 to 150 mg/L, *Salmonella* reductions were higher at 150 mg/L in the first 10 s of washing compared to the other FCC concentrations ( $p \leq 0.05$ ). For disinfection treatments with duration higher than 10 s, no statistical differences were detected between the reductions observed for treatments with FCC from 50 to 150 mg/L at a given time. These results provide evidence that increasing the FCC for treatments exceeding 10 s does not lead to an increase in chlorine disinfection efficacy at the evaluated conditions. This finding contributes to setting optimally operational conditions for chlorine washing in the fresh produce industry.

Overall, by increasing the washing duration greater *Salmonella* reductions were observed. The greatest *Salmonella* log-decrease was found in the first 20 s of washing (Fig. 1). The maximum mean reduction observed corresponded to 2.6 log after 2.5-min washings, evidencing that *Salmonella* was able to survive at all assayed FCC and exposure times. Based on the findings of prior research investigations, the efficacy of chlorine to inactivate microorganisms in fresh-cut produce is limited to 1-2 log reductions (Banach et al., 2017; Huang & Chen, 2018; Pablos et al., 2022). For example, 1.62 ± 0.38 log CFU/g reductions of *Salmonella* spp. were achieved on lettuce shreds during washing at 10 mg/L FCC for 2 min (Huang & Chen, 2018). In the study by Bovo et al. (2015), washing of fresh-cut lettuce at 70 mg/L FCC for 1 min, resulted in a

**Table 2**

*Salmonella* Typhimurium reductions (log CFU/cm<sup>2</sup>) during washing of fresh-cut lettuce with chlorinated water with free chlorine concentrations ranging from 50 to 150 mg/L for up to 150 seconds.

Time (s)	Free chlorine concentration (mg/L)			
	50	75	100	150
0	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>
5	1.02 ± 0.25 <sup>a</sup>	1.07 ± 0.28 <sup>a</sup>	1.66 ± 0.20 <sup>b</sup>	1.64 ± 0.13 <sup>b</sup>
10	1.10 ± 0.06 <sup>a</sup>	1.33 ± 0.14 <sup>a</sup>	1.62 ± 0.37 <sup>ab</sup>	2.29 ± 0.49 <sup>b</sup>
20	1.66 ± 0.29 <sup>a</sup>	1.65 ± 0.09 <sup>a</sup>	1.83 ± 0.14 <sup>a</sup>	1.98 ± 0.63 <sup>a</sup>
30	2.02 ± 0.35 <sup>a</sup>	1.72 ± 0.17 <sup>a</sup>	1.88 ± 0.33 <sup>a</sup>	2.33 ± 0.55 <sup>a</sup>
60	2.51 ± 0.44 <sup>a</sup>	2.34 ± 0.15 <sup>a</sup>	1.99 ± 0.11 <sup>a</sup>	2.32 ± 0.18 <sup>a</sup>
150	2.61 ± 0.14 <sup>a</sup>	2.47 ± 0.12 <sup>a</sup>	2.63 ± 0.21 <sup>a</sup>	2.47 ± 0.15 <sup>a</sup>

Mean reductions ± standard deviations are reported (n = 3).

Same superscript letters in the same row represent the homogeneous groups according to Tukey's multiple comparison of means (95% family-wise confidence level).

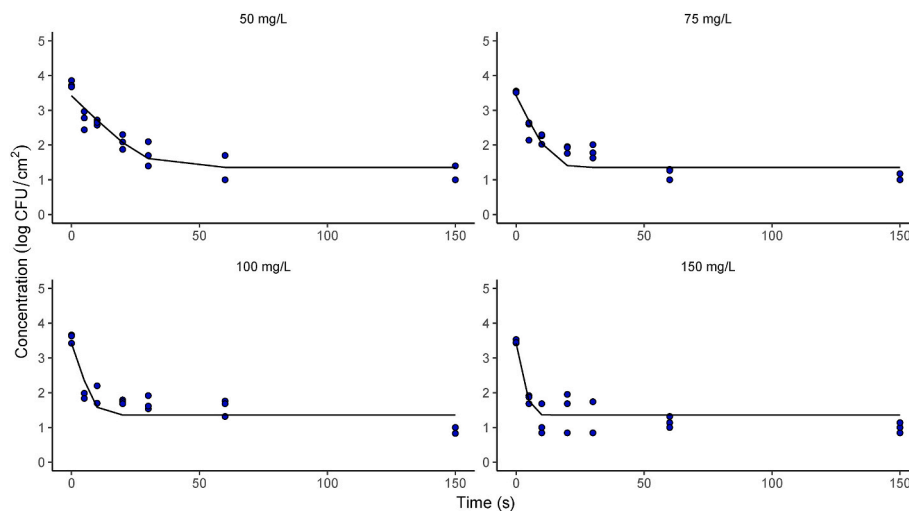


Fig. 1. Survival data of *Salmonella* Typhimurium in fresh-cut lettuce during chlorinated washing at different free chlorine concentrations (50–150 mg/L) for up to 150 seconds. (●) Observed data (—) Fittings of the combined Geeraerd and linear model to data.

*Salmonella* spp. reduction of  $1.28 \pm 0.14$  CFU/cm<sup>2</sup>. In our study, more than 2 log reductions were achieved during washing at this FCC-time combination (Table 2).

Differences between chlorine disinfection efficacy between studies may be related to the presence of lettuce exudates and organic matter (e. g., soil, dirtiness) on chlorinated solutions and on lettuce surface, besides factors related to experimental setups such as *Salmonella* strains evaluated. Exudates that are released from wounded lettuce tissues can react with free chlorine during washing, affecting its effectiveness to inactivate foodborne pathogens (Nou et al., 2011). Organic matter causes free chlorine depletion, reducing disinfection power, besides serving as vehicle for microbial contamination (Borges et al., 2020; Possas & Pérez-Rodríguez, 2023). In this sense, absorbance at 254 nm (UV254) and turbidity have been identified as relevant parameters for monitoring the organic matter load in wash water in real-time (López-Gálvez et al., 2019).

Results of previous investigations called attention on wash water disinfection to prevent cross-contamination between contaminated and noncontaminated produce (Banach et al., 2017; Van Haute et al., 2015). To assure the disinfection performance of the washing system, it is recommended to replenish water with chlorine during processing (Zhou et al., 2015). However, caution must be taken when adding chlorine to these systems, as higher chlorine levels can lead to increased formation of disinfection-by-products (DPBs), which could be present in both wash water and produce surface. The DPBs, such as trihalomethanes, are formed when chlorine reacts with organic matter in water, and have been associated with certain health concerns, including increased cancer risks (Villanueva et al., 2015).

Extensive investigations have been conducted to assess the combined effect of chlorine washing with other disinfection treatments, to identify approaches that can effectively reduce added chlorine levels, while achieving desired levels of microbial inactivation (Guo et al., 2017; Huang & Chen, 2018). For example, the application of pulse light with chlorine was more effective in inactivating *Salmonella* in lettuce shreds compared to chlorine washing independently (Huang & Chen, 2018). Furthermore, environmentally friendly alternatives to chlorine have shown high disinfection efficacy (Han et al., 2021). To illustrate that, water-assisted pulse light (at 0.15 and 0.30 J/cm<sup>2</sup>) and ultraviolet treatments (at 13 and 28 mW/cm<sup>2</sup>) for 1 or 2 min resulted in 1.9–3.1 log reductions of *Salmonella* spp. in lettuce (Huang & Chen, 2019). Combining the application of organic acids (i.e., malic, lactic and citric) at concentrations ranging from 0.3 to 2.0% with ultrasound treatments (frequencies of 40 kHz, power of 30 W/L) for 5 min, yielded *Salmonella* Typhimurium reductions of up to  $3.18 \pm 0.16$  log CFU/g on lettuce

(Sagong et al., 2011). Besides increasing the microbiological safety of lettuce, these technologies and applications minimize the use of hazardous chemical treatments during fresh-produce post-harvest processes, contributing to mitigate potential health risks.

### 3.2. Modelling microbial inactivation by chlorine washing in fresh-cut lettuce

The log-linear with tail model (Geeraerd et al., 2000) was fitted to the inactivation kinetics of *Salmonella* in fresh-cut lettuce obtained at different FCC. This model, which was proposed by Geeraerd et al. (2000), has been previously tested in a similar context to describe the inactivation kinetics of *E. coli* during washing of leafy-greens with peracetic acid and lactic acid solutions (Van Haute et al., 2015).

The data derived from experiments at 0 and 25 mg/L FCC were not included in modelling analysis, since no significant inactivation was observed at these concentrations, thus the model domain is 50–150 mg/L FCC, which is the range of concentrations most applied in fresh produce industry. By fitting the log-linear with tail model to the survival curves obtained at each FCC causing significant microbial inactivation, i. e., 50, 75, 100 and 150 mg/L- totaling four survival curves, it was noted that the  $\log_{10}N_0$  and  $\log_{10}N_{res}$  were rather homogeneous regardless of FCC concentration (Supplementary Table 1). Moreover, it was observed that the  $k_{max}$  estimated for the individual curves, increased linearly with the increase in FCC. Thus, it was more statistically suitable to fit the log-linear model (Equation (1)) coupled with a linear model describing the changes in  $k_{max}$  as a function of FCC (Equation (2)) to the whole set of inactivation data, which comprised a total of 84 observations.

The model parameters estimated through the global regression analysis are depicted in Table 3 and the model fitted to *Salmonella* data is shown in Fig. 1. Despite the high variability in the data (Fig. 1), the model showed an acceptable fitting, as indicated by the goodness-of-

Table 3

Results of the global regression analysis of the *Salmonella* Typhimurium inactivation data obtained during fresh-cut lettuce washing with free chlorine concentrations from 50 to 150 mg/L for up to 150 seconds.

Parameters	Estimate	Standard Error	t value	Pr (>t)
$\log_{10}N_0$ (log CFU/cm <sup>2</sup> )	3.4176	0.0882	38.74	0.0000
$\log_{10}N_{res}$ (log CFU/cm <sup>2</sup> )	1.3551	0.0519	26.09	0.0000
a (log CFU/(s.mg/L))	0.0069	0.0011	6.36	0.0000
b (log CFU/s)	-0.1811	0.0610	-2.97	0.0039

fitness measures  $RMSE = 0.34$  and  $R_{adj}^2 = 0.84$ . The high variability observed in the data is reasonable in disinfection treatments using chlorine and was also marked in other disinfection studies performed in our laboratory (Cuggino et al., 2020; Possas, Pérez-Rodríguez, et al., 2021). The  $\log_{10}N_{res}$  parameter reflects a tailing effect after a rapid decay on *Salmonella* populations at all FCC. This effect can be observed in Fig. 1, where it is evident that increasing the exposure times from 1 to 2.5 min did not yield an improvement in disinfection efficacy across all tested FCC. The tail in survival curves could be related to the presence of a fraction of resistant cells within the *Salmonella* population, which persists for longer washing times. However, further experiments should be performed to elucidate this hypothesis. The model resultant from coupling Equations (1) and (2) is shown in Equation (3).

$$\log_{10} N = 2.0625 * \exp(- (0.0069 * FCC - 0.1811) * t) + 1.3551$$

Equation 3

The linear model relating the  $k_{max}$  and the FCC (Equation (2), Table 3) is represented in Fig. 2. The estimated  $k_{max}$  for *Salmonella* at FCC from 50 to 150 mg/L varied from 0.17 to 0.86 log CFU/s. Note that the 95% confidence interval of model predictions is wider at higher FCC (Fig. 2). This is due to the propagation of uncertainty of the regression coefficients, which has a stronger influence on  $k_{max}$  at higher chlorine concentrations compared to lower ones.

To date, this is the first model relating the FCC in wash water with *Salmonella* inactivation rates in fresh-cut lettuce. The model by Cuggino et al. (2020) was developed following the response surface methodology, approximating the relationship between *Salmonella* inactivation levels during lettuce washing with free chlorine, exposure time and lettuce cutting size by a quadratic polynomial. Although this approach is valuable for understanding *Salmonella* dynamics during chlorine washing, it is worth noting that, in certain cases polynomial models may generate predictions that could be deemed unrealistic. Furthermore, Prado-Silva et al. (2015) developed a meta-analysis model predicting the reductions of *Salmonella* spp. in fresh produce as a function of sanitizer, sanitizer concentration, washing time and temperature, including chlorine-based solutions. While this approach synthesizes findings from numerous studies, it is essential to acknowledge that the heterogeneity in experimental setups across these studies can affect the validity and generalizability of the meta-analytical results.

The model developed in our study is especially relevant in the framework of quantitative risk assessments, providing a suitable prediction tool to incorporate the effect of specific chlorine concentrations in the risk by *Salmonella* in leafy greens. Different organic matter loads could be also addressed by the model, selecting, in the prediction variables, an equivalent level of free chlorine. Interestingly, the model could be recalibrated with data from similar washing context provided the same main factors are considered. Additionally, the model developed in our study reflecting the efficacy of a well established disinfection treatment may serve as basis for optimizing and assessing the effectiveness of more environmentally friendly and innovative disinfection strategies. This model has been already implemented in the predictive microbiology software MicroHibro ([www.microhibro.com](http://www.microhibro.com)) to be used for the evaluation of chlorine washing as a measure to control *Salmonella* in the fresh-cut lettuce industry.

#### 4. Conclusions

This study confirms the efficacy of chlorine for *Salmonella* inactivation in fresh-cut lettuce at free chlorine concentrations ranging from 50 to 150 ppm for up to 150 s. Chlorine washing alone was not able to eradicate *Salmonella* in fresh-cut lettuce, although greater reductions were achieved by increasing free chlorine levels in wash water. The log-linear with tail primary model combined with a linear secondary model was fitted to the survival data and the developed model was acceptable to predict *Salmonella* concentrations in fresh-cut lettuce as a function of

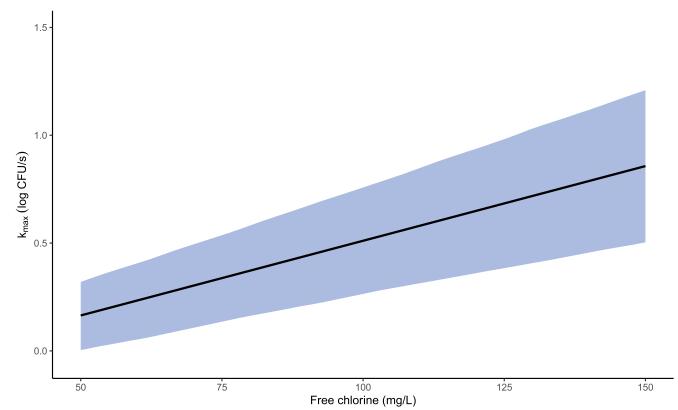


Fig. 2. Effect of free chlorine concentrations (mg/L) on *Salmonella* Typhimurium inactivation rates ( $k_{max}$ , log CFU/s) in fresh-cut lettuce. The blue zone represents the 95% confidence interval of model predictions.

washing times and free chlorine concentrations in wash water. This model has been already implemented in the user-friendly software MicroHibro ([www.microhibro.com](http://www.microhibro.com)) to be used for the decision-making process and the evaluation of chlorine washing as a control measure to increase the safety of fresh-cut lettuce with regards to *Salmonella*. Further, the model developed in this study can be used as a basis for the assessment and optimization of environmentally friendly alternatives to chlorine washing. Future studies must investigate the role of chlorine-induced viable but non-culturable state in microbial cells to access the safety of fresh-cut lettuce subjected to chlorine washing, concerning the presence of *Salmonella*.

#### CRedit authorship contribution statement

Arícia Possas: Investigation, Methodology, Data curation, Writing – original draft. Guiomar Denisse Posada-Izquierdo: Investigation, Methodology. Fatih Tarlak: Data curation, Writing – review & editing. Francisco Jiménez-Jiménez: Data curation, Writing – review & editing. Fernando Pérez-Rodríguez: Formal analysis, Visualization, Supervision, Writing – review & editing.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2023.115069>.

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