Food Research International 167 (2023) 112451

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Effects of chlorine and peroxyacetic acid wash treatments on growth kinetics of *Salmonella* in fresh-cut lettuce

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ARTICLE INFO

Keywords:
Predictive models
Growth rate
Bělehrádek model
Lettuce disinfection
Microbial risk assessment
Modified Atmosphere Packaging
Cross contamination
Internalization
Bacterial attachment

ABSTRACT

Fresh-cut produces are often consumed uncooked, thus proper sanitation is essential for preventing cross contamination. The reduction and subsequent growth of *Salmonella enterica* sv Thompson were studied in pre-cut iceberg lettuce washed with simulated wash water (SWW), sodium hypochlorite (SH, free chlorine 25 mg/L), and peroxyacetic acid (PAA, 80 mg/L) and stored for 9 days under modified atmosphere at 9, 13, and 18 °C. Differences in reduction between SH and PAA were non-existent. Overall, visual quality, dehydration, leaf edge and superficial browning and aroma during storage at 9 °C were similar among treatments, but negative effects increased with temperature. These results demonstrated that PAA can be used as an effective alternative to chlorine for the disinfection of *Salmonella* spp. in fresh-cut lettuce. The growth of *Salmonella enterica* sv Thompson was successfully described with the Baranyi and Roberts growth model in the studied storage temperature range, and after treatment with SWW, chlorine, and PAA. Subsequently, predictive secondary models were used to describe the relationship between growth rates and temperature based on the models' family described by Bělehrádek. Interestingly, the exposure to disinfectants biased growth kinetics of *Salmonella* during storage. Below 12 °C, growth rates in lettuce treated with disinfectant (0.010–0.011 log CFU/h at 9 °C) were lower than those in lettuce washed with water (0.016 log CFU/h at 9 °C); whereas at higher temperatures, the effect was the opposite. Thus, in this case, the growth rate values registered at 18 °C for lettuce treated with disinfectant were 0.048–0.054 log CFU/h compared to a value of 0.038 log CFU/h for lettuce treated with only water. The data and models developed in this study will be crucial to describing the wash-related dynamics of *Salmonella* in a risk assessment framework applied to fresh-cut produce, providing more complete and accurate risk estimates.

1. Introduction

Over the last decades, our society has changed its consumption patterns, leading to an increased demand for fresh, healthy, safe, and easy-to-prepare food products (Guo, Huang, & Chen, 2017; Yousuf, Deshi, Ozturk, & Siddiqui, 2020; del Carmen Rodríguez et al., 2017). Governments and organizations promote the consumption of fresh fruits and produce as a part of a healthy diet and to reduce the incidence of certain diet-related diseases (e.g., cardiovascular, obesity, etc.) (European Food Safety Authority, 2010; US Department of Health and Human Services, 2020). Therefore, fresh produce and minimally processed vegetables have gained popularity worldwide (Castro-Ibáñez, Gil, & Allende, 2017; Mir et al., 2018).

The consumption of fresh and freshly cut produce has been linked to several foodborne disease outbreaks as a consequence of the presence of several pathogenic bacteria, such as *Listeria monocytogenes*, *Clostridium botulinum*, *Bacillus cereus*, *Escherichia coli* O157:H7, and *Salmonella* spp. (Balali, Yar, Afua Dela, & Adjei-Kusi, 2020; Callejón et al., 2015; Centers for Disease Control and Prevention, 2019, European Food Safety Authority, 2017; Iwu & Okoh, 2019; Machado-Moreira, Richards, Brennan,

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https://doi.org/10.1016/j.foodres.2022.112451
Received 16 September 2022; Received in revised form 30 December 2022; Accepted 31 December 2022
Available online 26 January 2023

Abram, & Burgess, 2019).

The number of outbreaks associated with the consumption of contaminated fresh products has increased all over the world (Castro-Ibáñez, Gil, & Allende, 2017; European Food Safety Authority, 2013; Iwu & Okoh, 2019; Machado-Moreira, Richards, Brennan, Abram, & Burgess, 2019). Studies carried out in South America have reported the presence of *Salmonella* spp. in minimally processed vegetables (Gentili, Marzocca, Oriani, & Baldini, 2017; Gómez Albanys, Toledo Lisette, Quintero Giovanna, Donado Yadira, Roo Yeiny, & Leal Kutchynskaya, 2018; Maistro, Miya, Sant'Ana, & Pereira, 2012; de Oliveira, Maciel de Souza, Morato Bergamini, & De Martinis, 2011).

The growing trend might be related to an increase in the consumption of fresh produce, the contamination from livestock farming near crop areas, the rapid global availability of foodstuffs sometimes produced in areas with unknown hygienic conditions, and the increase of susceptible population groups, with a higher number of immunocompromised consumers (Beuchat, 2002; Olaimat & Holley, 2012).

Salmonella spp. can survive in soil and treated waters, as well as on fresh produce for long periods (i.e., in term of months), particularly at cold temperatures (European Food Safety Authority, 2014; Jacobsen & Bech, 2012). Controlling *Salmonella* spp. contamination requires a systematic approach that includes several aspects from farm to table: the quality of the raw material, the efficacy of the sanitation steps to prevent cross-contamination throughout the production chain, and appropriate storage temperatures (Castro-Ibáñez et al., 2017; Mir et al., 2018; Ssemanda et al., 2018).

Several researchers have emphasized the importance of washing and sanitizing treatments (Gil et al., 2015; López-Gálvez, Tudela, Allende, & Gil, 2019; Meireles, Giaouris, & Simões, 2016). A proper application of disinfection treatments could reduce pathogens in washing water, preventing cross-contamination (Banach, Sampers, Van Haute, & van der Fels-Klerx, 2015; López-Gálvez, Truchado, Tudela, Gil, & Allende, 2020; Maffei, Sant'Ana, Franco, & Schaffner, 2017).

Sodium hypochlorite (SH) is the most widely used disinfectant in the vegetable industry (Van Haute, Sampers, Holvoet, & Uyttendaele, 2013; Weng et al., 2016) due to its relatively low price, easy application, and wide spectrum of antimicrobial activity (Ramos, Miller, Brandão, Teixeira, & Silva, 2013). However, under certain conditions, this disinfectant has limited efficacy in reducing microbial loads because it is sequestered by organic matter and its action is highly pH-dependent (Chen & Hung, 2017; Cuggino et al., 2020; Waters & Hung, 2014; Weng et al., 2016).

In addition, it has been found that, during the washing process, harmful disinfection byproducts (DBPs) can be formed (López-Gálvez et al., 2019; T. Zhang, Lee, Luo, & Huang, 2022). Besides having potential adverse consequences on environment and human health (Lee & Huang, 2019; Simpson & Mitch, 2021), these substances cause unpleasant flavor and odor in fresh produces, resulting in negative sensory responses (Gil, Gómez-López, Hung, & Allende, 2015; Gómez-López, Lannoo, Gil, & Allende, 2014; Van Haute et al., 2013).

Different approaches to reduce or replace the use of chlorine have already been developed, including biological methods, alternative chemical compounds, and physical technologies, as well as combinations thereof (Birmpa, Sfika, & Vantarakis, 2013; Meireles et al., 2016; Pablos et al., 2018; Petri, Rodríguez, & García, 2015; S. Van Haute et al., 2015).

In this regard, peroxyacetic acid (PAA) has been proposed as a potential alternative to chlorine, as an antimicrobial capable of reducing microorganisms on fresh produce (Fallik, 2014; López-Gálvez et al., 2020; Osaili, Alaboudi, Al-Quran, & Al-Nabulsi, 2018; P. Singh, Hung, & Qi, 2018). PAA is less sensitive in the presence of organic matter than sodium hypochlorite and does not produce harmful disinfection byproducts (Lee & Huang, 2019; Lippman, Yao, Huang, & Chen, 2020; Zoellner, Aguayo-Acosta, & Dávila-Aviña, 2018), thus resulting in a low environmental impact (Davidson, Kaminski-Davidson, & Ryser, 2017; Van Haute et al., 2015).

It is worthy to highlight that processing fresh produce does not completely eliminate microbial contamination. This means that it is also important to select a correct packaging and controlling the time and temperature along the distribution and consumption chains to prevent growth of both pathogens and spoilage microorganisms (Castro-Ibáñez et al., 2017; de Frias et al., 2018; Luo, He, & McEvoy, 2010).

Packaging plays an relevant role in the microbiological protection of fresh-cut produces (Turatti, 2011; World Health Organization/Food and Agriculture Organization, (WHO/FAO), 2008). In this sense, it is determinant the selection of the packaging material, the conditions that the packaging generates and the relationship of weight/volume between product and packaging. Modified atmosphere packaging (MAP) has been introduced as an enhancement technology to extend the shelf life of Ready to Eat (RTE) vegetables (Oliveira et al., 2010; Posada-Izquierdo, Zurera, & Pérez-Rodríguez, 2014; Ramos, Miller, Brandão, Teixeira, & Silva, 2013; Zhuang, Barth, & Cisneros-Zevallos, 2014). Mainly, it provides a reduced partial pressure of O_2 to retard browning and inhibits or delays the growth of spoilage microorganisms and foodborne pathogens (Horev et al., 2012; Jideani, Anyasi, Mchau, Udoro, & Onipe, 2017; Paillart et al., 2017).

The recommended temperature to ensure ready-to-eat vegetables quality and safety is 4 °C (Food and Drug Administration, 2008; Rediers, Claes, Peeters, & Willems, 2009; Food and Drug Administration, 2012), even though a high percentage of domestic and commercial refrigerators do not meet this temperature criterion (Andritsos, Stasinou, Tserolas, & Giaouris, 2021; Atilio de Frias, Luo, Kou, Zhou, & Wang, 2015; Jofré, Latorre-Moratalla, Garriga, & Bover-Cid, 2019; Jovanovic, Djekic, Smigic, Tomic, & Rajkovic, 2022; Ovca, Škufca, & Jevšnik, 2021; Tsironi et al., 2017).

Although numerous small-scale laboratory studies have been performed to assess the efficacy of disinfectants against pathogens on leafy greens (Beuchat, Adler, & Lang, 2004; Huang & Chen, 2011; Keskinen, Burke, & Annous, 2009; Keskinen & Annous, 2011), little has been done on the influence of the use of PAA and chlorine, as bactericidal agents, on the subsequent steps of the cold chain.

According to Ndraha, Goh, Tran, Chen, and Hsiao (2022), the estimation of microbial growth kinetics considering the food-specific characteristics and environmental conditions under which foods are manufactured and stored is a need for quantitative microbiological risk assessment (QMRA). Consequently, it is necessary to collect information on the growth kinetics of specific pathogens in fresh vegetables, considering different processing conditions and treatments.

The aim of this work was to quantitatively compare the disinfection efficacy of three sanitizing water treatments (water, SH, and PAA) against *Salmonella* on cut iceberg lettuce and its subsequent growth potential at different storage temperatures (9, 13, and 18 °C), using a predictive modeling approach.

2. Materials and methods

2.1. Fresh-cut lettuce sample preparation

Unprocessed heads of iceberg lettuce (*Lactuca sativa* L.) were obtained from a local market in Córdoba (Spain). The samples were selected, from the refrigerated shelves (4 °C), according to their packaging date, which was usually the same as the harvest date. The transfer to the laboratory was carried out in coolers with ice, to keep the samples refrigerated. After its reception in the laboratory, lettuce was stored at 4 °C and used within 2 h upon arrival in the laboratory in order to avoid any additional microbial and sensory deterioration. The core and leaves of damaged lettuce were manually removed. The internal leaves were cut into commercial sized pieces of 3 × 3 cm (9 cm^2) under aseptic conditions. Sample processing was carried out in a refrigerated room (4 °C), simulating industrial conditions for the production of fresh-cut lettuce.

2.2. Bacterial strain, growth conditions and inoculum preparation

Salmonella enterica sv Thompson pGT-Kan mB156 gentamicin-resistant, labeled with green fluorescent protein (GFP), was used in this study to avoid potential interferences from endogenous microbiota, allowing for a more precise and accurate enumeration of the inoculated pathogen. Cryoculture reactivation was carried out in Brain Heart Infusion (BHI, Oxoid, UK) and three consecutive subcultures were performed. Subsequently, cells from the last subculture were washed by centrifugation (Jouan C4i, Thermo Electron Corporation, France) at 4100 rpm for 10 min with a phosphate buffer (PBS, Medicago, Sweden). The pellet was resuspended in PBS and placed onto Plate Count Agar (PCA, Oxoid, UK) supplemented with gentamicin (15 μg/mL) and incubated at 37 °C for 24 h to obtain live culture. A colony of the live culture was streaked on PCA + gentamicin plate, incubated at 37 °C for 16 h, and refrigerated (<7 °C) until experiments were conducted. The inoculum was prepared by adding the colonies into a saline solution (0.85 %, w/v) (Merck, Germany) up to approximately $2x10^7$ CFU/mL. The concentration was initially adjusted using absorbance at 600 nm in a Bioscreen C analyzer (Labsystems, Helsinki, Finland) and then confirmed by plate count. Serial dilutions of the *Salmonella* inoculum were carried out to obtain the desired range of bacterial concentrations of *ca.* 5, 6 and 7 log CFU/mL.

The inoculation process was carried out on sterilized trays where each lettuce piece was individually inoculated with 0.1 mL *Salmonella* cell suspension in saline solution. The inoculum was spread on the lettuce piece homogeneously with a micropipette. The samples were then maintained at 12 °C and 60 % RH for 45 min to enable the drying of the inoculum suspension with bacteria attached to vegetable tissues.

The lettuce samples treated with disinfectant would result in lower *Salmonella* concentrations than those obtained in the experiments with only water, generating distinct starting microbial levels in the storage assay. As it could influence pathogen kinetics, in order to minimize its effect, the initial inoculum concentration for samples to be treated with only water was adjusted to one logarithm lower than that used in the experiments with disinfectant.

2.3. Disinfection treatments

2.3.1. Simulated industrial process water

Simulated wash water (SWW) was prepared based on the composition of water used in the Spanish vegetable processing industry following the procedure reported by Pablos et al. (2018) and Cuggino et al. (2020). Briefly, it consisted of formulating different chemical agents (ionic compounds, kaolin powder, malt extract, among others) in sterilized distilled water in order to reproduce the typical values of the main physicochemical parameters registered in industrial process water. The values for pH (6.5), oxidation/reduction potential (530 mV), Total Organic Carbon (TOC 150 mg/L), turbidity (100 NTU (Nephelometric Turbidity Unit), conductivity /1050 μS/cm), total dissolved solids TDS (750 mg/L) and temperature were measured using the Multi-Parameter PCS Testr 3.5 (Oakton, USA) to monitor the established industrial parameters in order to verify that the model water processes were similar to those registered in the industry.

2.3.2. Simulation of the lettuce washing process

The inoculated lettuce pieces were immersed in a sterile plastic container with SWW. The water: lettuce ratio corresponded to 8.5 L/kg, being equivalent to the industrial ratio used for these products (Cuggino et al., 2020; Pablos et al., 2018). Three types of treatments were tested, corresponding to SWW without sanitizer, and washing water formulated with SH or PAA.

For the disinfection treatments, the final concentration was 25 mg/L and 80 mg/L of free chlorine (sodium hypochlorite, Sigma–Aldrich, USA) and PAA solution (Merck, Germany), respectively. The disinfectant levels were chosen on an industrial basis, and considering previous works, in which sensory impact was also evaluated (Beuchat, Adler, & Lang, 2004; Cuggino et al., 2020; Lippman, Yao, Huang, & Chen, 2020; Osaili, Alaboudi, Al-Quran, & Al-Nabulsi, 2018; Code of Federal Regulations CFR, 2012; Singh et al., 2018). The pH of the chlorinated water was adjusted to 6.5 with a solution of 0.1 M HCl (Merck, Germany).

The free chlorine concentration and pH of the chlorinated water were monitored in the wash tanks using a HI93734 meter (Hanna Instruments, UK).

The samples were treated for 60 s under constant agitation at 4 °C. The disinfection process (i.e., oxidizing effect) was halted at 60 s by adding 180 μL Sodium Thiosulphate (Sigma-Aldrich, USA), as a neutralizer, into the wash tank, avoiding that bacterial inactivation could extend beyond the time defined in the experimental set-up for washing (López-Gálvez, Allende, Selma, & Gil, 2009; S. Van Haute et al., 2015). The samples were drained with a manual centrifuge (3.5 L) (model 23,200 Leifheit, Germany) to remove excess water.

2.3.3. Packaging and storage conditions

The processed lettuce samples were aseptically packaged into individual sterile plastic bags, which were perforated, producing 4 holes Ø2 mm. The dimensions of the bags were 7 × 5 × 1 cm according to the weight/volume ratio used for commercial bags (250 g/1.38 L). Triplicates of packaged treated samples were placed in anaerobic jars (3.5 L) with the atmosphere generation sachet CampyGen (Thermo Scientific, Oxoid, Japan). As plastic bags were perforated, samples were exposed to the atmosphere generated in the jar, whose gas composition was: 85 % N_2, 10 % CO_2, 5 % O_2. These values are in line with the values registered in commercial packages for this type of vegetables. Packaged samples were stored at 9, 13, and 18 °C for 9 days. The temperatures are within the growth range of *Salmonella* and were selected because they represent potential scenarios of abuse of refrigeration temperature (9 °C and 13 °C), and environmental temperature (18 °C) in households, supermarkets and markets. The storage temperature was monitored with the MicroLite data logger (Fourier Technologies, Israel). The storage time was chosen based on the shelf life reported by manufacturers of these products. Samples were extracted for analysis on day 0 (the day experiments were performed) 3, 5, 7 and 9. At each sampling point, samples from each treatment were analyzed for sensory properties and *Salmonella* counts.

2.4. Microbiological analysis

The treated, untreated and packaged lettuce samples were microbiologically analyzed to enumerate *Salmonella*. For this purpose, lettuce pieces (~0.81 g) were placed into sterile tubes with 7.3 mL of 0.1 % peptone water (PW, Oxoid, UK), and vigorously shaken by vortexing for 30 s (Vortex mixer, ZX3, VELP Scientifica Srl, Italia) under aseptic conditions. Homogenized samples were serially diluted in saline solution (0.85 % NaCl) and plated in PCA supplemented with gentamicin to enumerate and identify the green fluorescence colonies of *Salmonella*. Microbiological counts of *Salmonella* were expressed as decimal logarithms of colony forming units (CFU) per gram (log CFU/mL). Each experiment was performed with three replicates of lettuce pieces and independently repeated three times.

2.5. Scanning electron microscopy analysis

Lettuce leaf pieces were incubated with different concentrations of *Salmonella* (ca. 4–7 log CFU/mL) at 25 °C for 1 h, in the presence of light, following the inoculation procedure described above (*section 2.2*). A subset of lettuce leaf pieces was then washed with water, simulating the washing process, following the same protocol as above (*section 2.3.2*). Internal 1 × 1 mm squares of treated lettuce samples were then excised under sterile conditions and fixed at 7 °C for 24 h in 2 % glutaraldehyde (Alfa Aesar, Germany). After that, samples were dehydrated in 30 %, 50 %, 70 %, 90 %, and 100 % absolute acetone (Scharlau, Spain). They

were then critical-point dried with CO_2, mounted on a stand, and sputter-coated with a thin layer of gold in a high vacuum condition. Digital images were captured with a JOEL JSM 7800F scanning electron microscopy (SEM) (JOEL, Japan).

2.6. Imaging analysis and sensory assessment of lettuce samples

2.6.1. Image acquisition

To analyze the visual quality of the treated and stored lettuce, digital photographs were taken of samples during storage. The images were obtained using a digital camera (Canon EOS 1300D, USA). The camera was mounted on a stand adjusted to 30 cm above the base. To avoid capturing shadows and glare in the photographs, four fluorescent lights (27 W) were placed at different points, then the lettuce pieces were placed on a gray board and photographed. All experiments were conducted in a dark room at room temperature. The camera was set to focal length of 55.0 mm on automatic indoor focus with the flash off. These settings provided a close-up view of the lettuce and covered the entire field of the sample. Three processed and packaged lettuce samples were photographed for each treatment and sampling time during storage at different temperatures. The captured pictures were exported to JPEG format for examination.

2.6.2. Sensory analysis

The sensory quality of the products after washing with and without different sanitizers was evaluated during storage by a sensory panel formed by 10 trained panelists, members of the Department of Bromatology and Food Technology of University of Cordoba and the Agri-Food Quality Transfer Center of the Faculty of Agricultural Sciences of the UNC. The sensory attributes evaluated corresponded to *Overall visual quality* (OVQ) (freshness and brightness), *Dehydration* (succulence and freshness), *Leaf edge browning,* and *Leaf superficial browning* and *Aroma (*identified as off-odor) using a category test with modifications (Lopez-Galvez, Ragaert, Palermo, Eriksson, & Devlieghere, 2013; Salgado, Pearlstein, Luo, & Feng, 2014; Zhang & Yang, 2017; Zhou et al., 2004). The sensory panel was trained to align with and adopt the descriptions for the sensory attributes proposed by Baur, Klaiber, Hammes, and Carle (2004) and de Oliveira, Leal, Honório, and Soares (2013). During training, the concept of each attribute was introduced through photos and lettuce samples that were selected according to the points of the scale used. The above attributes were scored with a 9-point scale as shown in Table 1.

Table 1
Definitions of the sensory attributes evaluated by the sensory panel and their corresponding sensory scores.

Attributes	Definitions	Sensory scores
Overall visual quality (OVQ)	Bright green color of fresh lettuce	1: absence of brightness/ opaqueness/staling, 3: a little, 5: moderate, 7: a lot, 9: presence of brightness/shininess
Aroma	Absence of unpleasant or strange odor	1: severe off-odor, 3: strong off-odor, 5: moderate off-odor, 7: a little off-odor, 9: no off-odor
Leaf edge browning	Appearance of browning in edges	1: Severe browning (70–100 % of edges browning), 3: moderately severe (50–70 % of edges with browning), 5: moderate (50 % of edges browning), 7: slight (<30 % of edges browning), 9: No browning
Leaf superficial browning	Appearance of browning in the midrib and surface	1: Severe browning (70–100 % of superficial browning), 3: moderately severe (50–70 % of superficial with browning), 5: moderate (50 % of superficial browning), 7: slight (<30 % of superficial browning), 9: No browning
Dehydration	Loss of succulence and turgidity of leaf, indicative of freshness	1: Dry and flaccid, 3: severe dehydration and loss of succulence, 5: moderate dehydration, 7: mild dehydration and good succulence, 9: hydrated and succulent

2.7. Growth model and kinetic parameter estimates

Salmonella counts were transformed into a decimal logarithmic scale and entered into Excel (Excel® 2010, Microsoft, Redmond/WA). The primary growth model of Baranyi & Roberts (1994) was fitted to growth data using the Excel DMFit 3.5 add-in (Institute of Food Research, Norwich, United Kingdom). Three kinetic parameters were estimated, namely, lag time (λ, expressed in h), maximum growth rate (μ_{max}, expressed in log CFU/h) and maximum population density (*MPD*, expressed in log CFU/g). The *MPD* parameter represents the upper asymptote of the predicted growth curve; however, some curves did not reach this asymptote. In these cases, it was calculated as the maximum predicted concentration (N_{max}, expressed in log CFU/g).

Secondary predictive models were also developed to represent the effect of temperature on μ_{max}, based on the family of models described by Bělehrádek (1926) represented by Eq. (1).

$$r = b \bullet (T - T_0)^m \quad (1)$$

In this model, r is a rate, b and T_0 are regression parameters, and T is temperature in °C. This equation was later applied by Ratkowsky, Olley, McMeekin, and Ball (1982) and in other works (Ross, 1987, 1993) for microbial growth using $m = 2$ and $r = \sqrt{\mu_{max}}$, with μ_{max} being the maximum growth rate, and interpreting T_0 as the notional minimum temperature for microbial growth, which is usually 5–10 °C lower than the actual minimum temperature. This model is extensively used in predictive microbiology as a secondary model. Further, the works by Dantigny (1998) and Dantigny and Molin (2000) reported that m values can range from 1 to 2, depending on whether the microorganism is mesophilic or psycrotrophic, respectively. In this work, Eq. (1) was fitted to data, using $m = 1$ and 2 and $r = \sqrt{\mu_{max}}$ and $ln(\mu_{max})$. The performance of the developed predictive models was evaluated using the Coefficient of Determination (R^2) and Standard Error (SE).

2.8. Data treatment and statistical analysis

The statistical analysis was performed using InfoStat (Grupo InfoStat, Argentina) (Di Rienzo et al., 2017). *Salmonella* reduction was calculated as the difference, on a logarithmic decimal scale, between bacterial counts in lettuce before treatment (N_0, log CFU/g) and after treatment (N_f, log CFU/g). In addition, the survival and growth of *Salmonella* in cut, treated, and stored lettuce were studied. The growth potential (δ) of *Salmonella* was determined by the difference between the microbial counts at the end (day "9″) (log CFU/g) and at the beginning (time "0") (log CFU/g) of shelf life (Beaufort, 2011). Three replicates per measurement were performed and data were expressed as the mean of replicates. Differences between means were determined using Ducan's test and Tukey's HSD (honestly significant difference) test with a confidence level of 95 % ($P < 0.05$).

3. Results

3.1. Effect of initial inoculum concentration on the reduction of Salmonella in fresh-cut lettuce during washing

The effect of initial inoculum concentration on *Salmonella* reduction in lettuce pieces after washing with SWW was studied using three different inoculum levels (*ca.* 4–6 log CFU/g). The positive correlation reported by the Spearman correlation coefficient (0.90, $p < 0.0001$)

indicated that *Salmonella* reduction increased significantly as the initial concentration of the inoculum increased. For graphical analysis, Fig. 1 illustrates the initial inoculum concentrations vs the reduction obtained at the end of the water treatment. The trend exhibited by the data points evidences the positive association shown by the Spearman correlation coefficient. Moreover, a statistical comparison of the reductions at different inoculum levels confirmed that the reductions were significantly ($p < 0.05$) higher (1.64 ± 0.32 CFU/g) in lettuce pieces contaminated with initial inoculum > 5.5 log CFU/g. In contrast, *Salmonella* inocula of 4.5–5.5 log CFU/g and lower than 4.5 log CFU/g resulted in lower reductions, corresponding to 1.04 ± 0.12 and 0.96 ± 0.04 log CFU/g, respectively, which were not statistically different ($p > 0.05$).

The study by Van der Linden et al. (2016) observed that higher inoculum levels were associated with greater reductions of *E. coli* O157:H7 and *Salmonella* populations in the cut edges and the surface region of iceberg lettuce pieces. On the contrary, the leaf pieces inoculated with a low inoculum showed a smaller decrease in cells after washing, which was most clearly noted in the cut edges. This behavior was also reported by other study, for *E. coli* O157:H7, which presented a greater attachment to cut edges of leaves, especially at lower inoculum levels (Takeuchi & Frank, 2001). In turn, large population densities cause cells to colonize sites other than wounds, stomata, cracks, and broken trichomes, presenting a lower attachment capacity (Takeuchi & Frank, 2001) that could facilitate washing-induced removal.

In line with the above, SEM images obtained from the analyzed lettuce samples suggest that, at higher levels, *Salmonella* covered a larger surface area of leaf tissues, showing a greater presence of clustering compared to lower levels (Fig. 2). In addition, the fact that experiments were performed under light conditions allowed stomata to be open, causing *Salmonella* to colonize near and inside the leaf stomata, as reported on other studies (Golberg, Kroupitski, Belausov, Pinto, & Sela, 2011; Kroupitski et al., 2009). The detachment of the most densely populated cell clusters on the flat and intact surface (cuticle) of leaf tissue could lead to a greater loss of *Salmonella* cells (Bermúdez-Aguirre & Barbosa-Cánovas, 2013; Takeuchi & Frank, 2001).

3.2. Salmonella reductions in fresh-cut lettuce washed with water and disinfectant solutions

The reductions on *Salmonella* populations in fresh-cut lettuce samples obtained with the different washing treatments are shown in Table 2. The initial inoculum size differed for each treatment in order to obtain similar final levels after treatment (i.e., the initial level for the storage phase). With this strategy, the possible influence of the initial concentrations on pathogen growth during the storage experiments was minimized (Coleman, Tamplin, Phillips, & Marmer, 2003; López-Gálvez, Gil, & Allende, 2018; Ma, Li, & Zhang, 2016). Thus, lettuce samples treated only with SWW were inoculated with a lower inoculum, corresponding to 4.6 log CFU/g, and samples treated with disinfectants were inoculated with inoculum levels of around 6.2 log CFU/g (Table 2). Nevertheless, according to the results provided in the previous section, which demonstrate an influence of the initial inoculum on *Salmonella* reductions, the effect thereof was deemed less relevant compared to the reduction caused by the different disinfectants used.

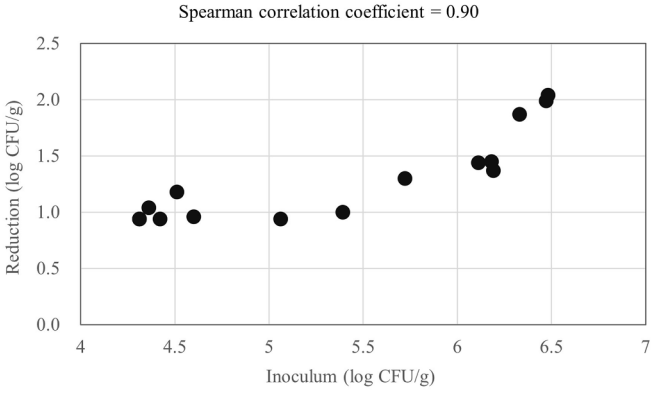

Fig. 1. Initial inoculum concentration vs reduction of *Salmonella* populations in fresh-cut lettuce (log CFU/g) at the end of the treatment with Simulated wash water (SWW) during 60 s.

Salmonella populations were reduced by 0.91 ± 0.07 log CFU/g after washing cut lettuce samples with SWW for 60 s under agitation. The effect of wash water on microbial reductions reported by other studies using similar treatment times ranged from 0.4 to 1.6 log CFU/g (Cap et al., 2020; Huang & Chen, 2018; Li et al., 2017; Lippman et al., 2020; Neal et al., 2012; Pahariya, Fisher, & Choudhary, 2022). In contrast, the effect of incorporating 25 mg/L of SH and 80 mg/L PAA caused a decrease in *Salmonella* contamination by 2.98 ± 0.19 and 2.79 ± 0.36 log CFU/g, respectively (Table 2). These values were significantly higher than those obtained with water alone ($p < 0.05$), but statistically similar between disinfectants ($p > 0.05$). The work by Huang, de Vries, and Chen (2018) reported no significant differences between both treatments with PAA and SH. In line with our study, these authors observed that the use of these chemicals significantly increased the reduction of *Salmonella* in relation to the control treatment, composed only of SWW.

Similar reductions were reported in a previous work conducted in our laboratory (Cuggino et al., 2020) and by other authors (Osaili et al., 2018; Pezzuto et al., 2016; Stopforth, Mai, Kottapalli, & Samadpour, 2008; Van Haute et al., 2013), but applying concentrations of disinfectant of 100–200 mg/L free chlorine. Pezzuto et al. (2016) found that washing raw rocket with 200 mg/L sodium hypochlorite reduced *Salmonella* counts by 2 logarithms.

For PAA, the results of our study indicated a greater *Salmonella* reduction compared to other studies (Lippman et al., 2020; Ruiz-Cruz, Acedo-Félix, Díaz-Cinco, Islas-Osuna, & González-Aguilar, 2007). For instance, Ruiz-Cruz et al. (2007) showed that washing shredded carrots reduced *Salmonella* populations by 2.1 log CFU/g; however, the product and disinfection values used were different, i.e., 40 ppm PAA and 2 min agitation. In shredded iceberg lettuce, *Salmonella* was reduced by 1.52 log CFU/g when samples were exposed to 80 ppm PAA for 2 min (Lippman et al., 2020). In a similar study (Banach et al., 2020) applied to fresh-cut lettuce, *E. coli* reductions of 2.8–3.0 log CFU/g were obtained using 80 mg/L PAA, for 2 min at 4 °C, equivalent to the values used in our study.

The differences in effectiveness found between studies, for both SH and PAA, are the result of several factors, namely food type, initial pathogen concentration, temperature, organic load, product to disinfectant solution ratio, type of washing treatment, pH of treatment solutions, concentration and disinfection time (Marçal, Campos, & Pintado, 2022; Pahariya et al., 2022).

3.3. Sensory quality during storage of fresh-cut lettuce washed with water and disinfectant solutions

The overall visual quality (OVQ) of the lettuce samples after processing (day 0) was scored by panelists at a mean of 7.91 ± 1.19, 8.38 ± 0.94, and 8.04 ± 1.19 for products treated with SWW, SH, and PAA, respectively (Fig. 3); indicating good brightness and freshness of lettuce samples, without significant differences between treatments. The relatively high standard deviation obtained in each group could be mostly due to the inherent variability of the processed lettuce leaf pieces, although the panelist assessment could be another relevant source of uncertainty in the scores. These results are in accordance with previous works, in which similar OVQ scores were observed between washing treatments comparable to those evaluated in this study (Lopez-Galvez et al., 2013; Salgado et al., 2014).

All the attributes studied in the samples stored at 9 °C were similar among treatments during storage ($p > 0.05$). However, when the

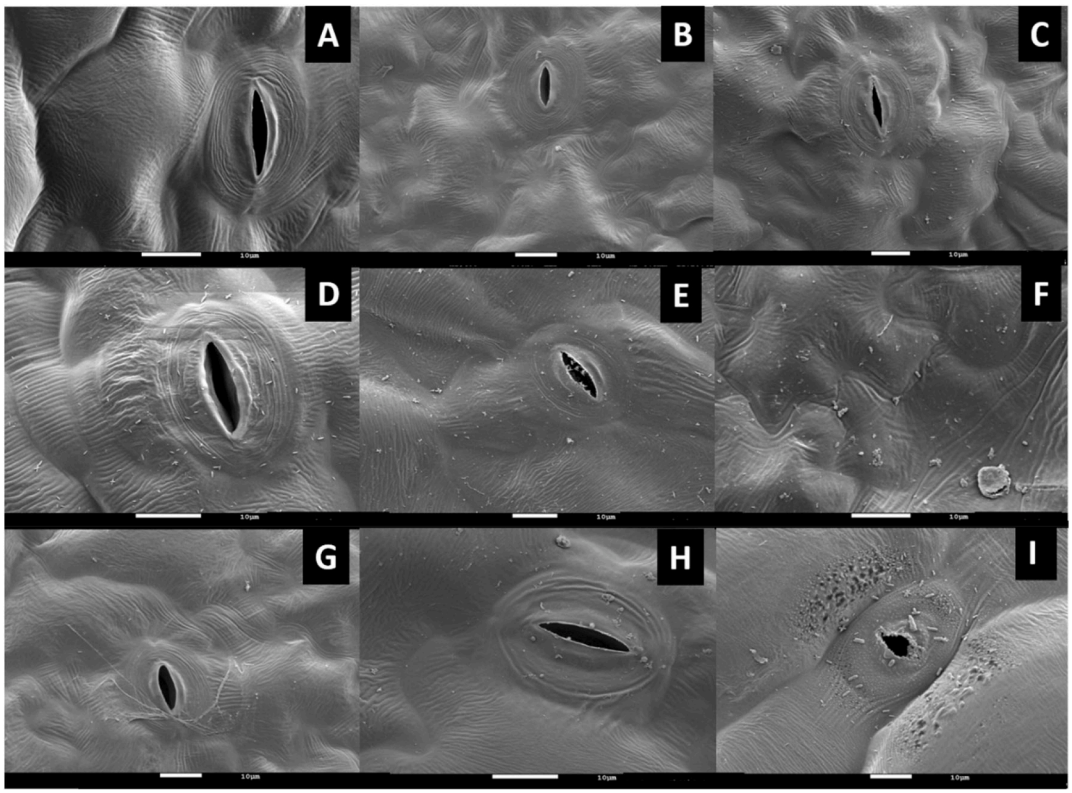

Fig. 2. Scanning electron microscope (SEM) images of *Salmonella* cells on lettuce leaf surfaces of control samples (A), untreated samples inoculated at 4.4 log CFU/g (B), 5.5 log CFU/g (C), 5.3 log CFU/g (D), 6.5 log CFU/g (E) and 6.2 log CFU/g (F) and samples treated with simulated wash water (60 s) resulting in final levels of 3.5 log CFU/g (G), 4.5 log CFU/g (H), and 4.2 log CFU/g (I).

Table 2
Salmonella reductions in fresh-cut lettuce washed with only Simulated wash water (SWW), SWW with 25 mg/L chlorine (SH) and SWW with 80 mg/L peroxyacetic acid (PAA).

Treatment	N_o[1] (log CFU/g)	N_f[2] (log CFU/g)	Reduction (log CFU/g)
SWW	4.61 ± 0.35	3.71 ± 0.40[3]	0.91 ± 0.07[2]
SH	6.28 ± 0.13	3.29 ± 0.10[1]	2.98 ± 0.19[1]
PAA	6.06 ± 0.45	3.27 ± 0.15[1]	2.79 ± 0.36[1]

[1] Initial concentration of *Salmonella* in fresh-cut lettuce.
[2] Concentration of *Salmonella* in fresh-cut lettuce after washing with water or a disinfectant solution during 60 s.
[3] Different letters in the same column indicate significant differences ($p < 0.05$) according to Tukey's test.

temperature increased, negative effects were observed on all attributes, which was more evident at 18 °C. In this respect, other authors have reported that the combination of disinfectants and storage at high temperatures increases the respiration rate of fresh-cut lettuce and water loss, resulting in a general deterioration of the lettuce pieces (Guan, Huang, & Fan, 2010; Luna et al., 2013; Vandekinderen et al., 2009).

Browning on samples stored at 18 °C was lower on the edges and surface of lettuce pieces treated with PAA compared to those disinfected with SH (Fig. 3). This result may be due, as indicated by Vandekinderen et al. (2009), to an increased respiration rate induced by SH in fresh-cut iceberg lettuce, whereas the PAA effects would be less relevant. It is important to note that browning in lettuce pieces can be caused by several factors. Environmental stress, temperature, and vegetable tissue damage, as well as the use of oxidizing agents for disinfection can trigger an increase in the activity of phenylalanine ammonia lyase (PAL). Upon PAL activity induction, phenolic compounds continue to accumulate. Such molecules are natural substrates for oxidative enzymes such as polyphenol oxidase (PPO) and peroxidase (POD), which give rise to quinones that polymerize producing brown pigments and discoloration at the cut edges of lettuce (García, Gil, & Tomás-Barberán, 2019; Hunter et al., 2017; Liu et al., 2021; Taranto et al., 2017). Furthermore, as mentioned by García et al. (2019), it is accepted that browning development could be affected by both the constitutive phenolic compounds (limited or absent in lettuce midrib tissues) that are substrates of PPO and those synthesized by PAL as a response to the wound signal. This could explain the lower browning found, in this work, on the lettuce surface, with respect to that on the edges.

The treatments evaluated in this work did not produce any anomalous or unpleasant odor (data not shown), which was in line with the observations reported by Lopez-Galvez et al. (2013) and Zhang and Yang (2017).

In general, the panelists' assessment apparently showed a direct correlation between OVQ and lettuce browning and dehydration, showing better results in the samples treated with PAA and SWW than in those treated with SH. In this regard, it is important to highlight that, at the time of purchase, consumers base their choice mainly on the OVQ, a combination of quality features that can be judged through the package (James, Ngarmsak, & Rolle, 2010).

3.4. Salmonella growth potential in fresh-cut lettuce washed with water and disinfectant solutions

The growth capacity of *Salmonella* in lettuce after washing only with SWW, SH and PAA for 60 s was evaluated at three different temperatures, 9, 13, and 18 °C, for 9 days under modified atmosphere conditions. The initial *Salmonella* concentration at the beginning of storage in fresh-cut lettuce was 3.55 ± 0.34 log CFU/g, 3.24 ± 0.14 log CFU/g, and 3.26 ± 0.14 log CFU/g for the samples treated with SWW, chlorine and PAA, respectively. At the end of the storage period (9 days), *Salmonella* concentrations increased at all storage temperatures, as shown in

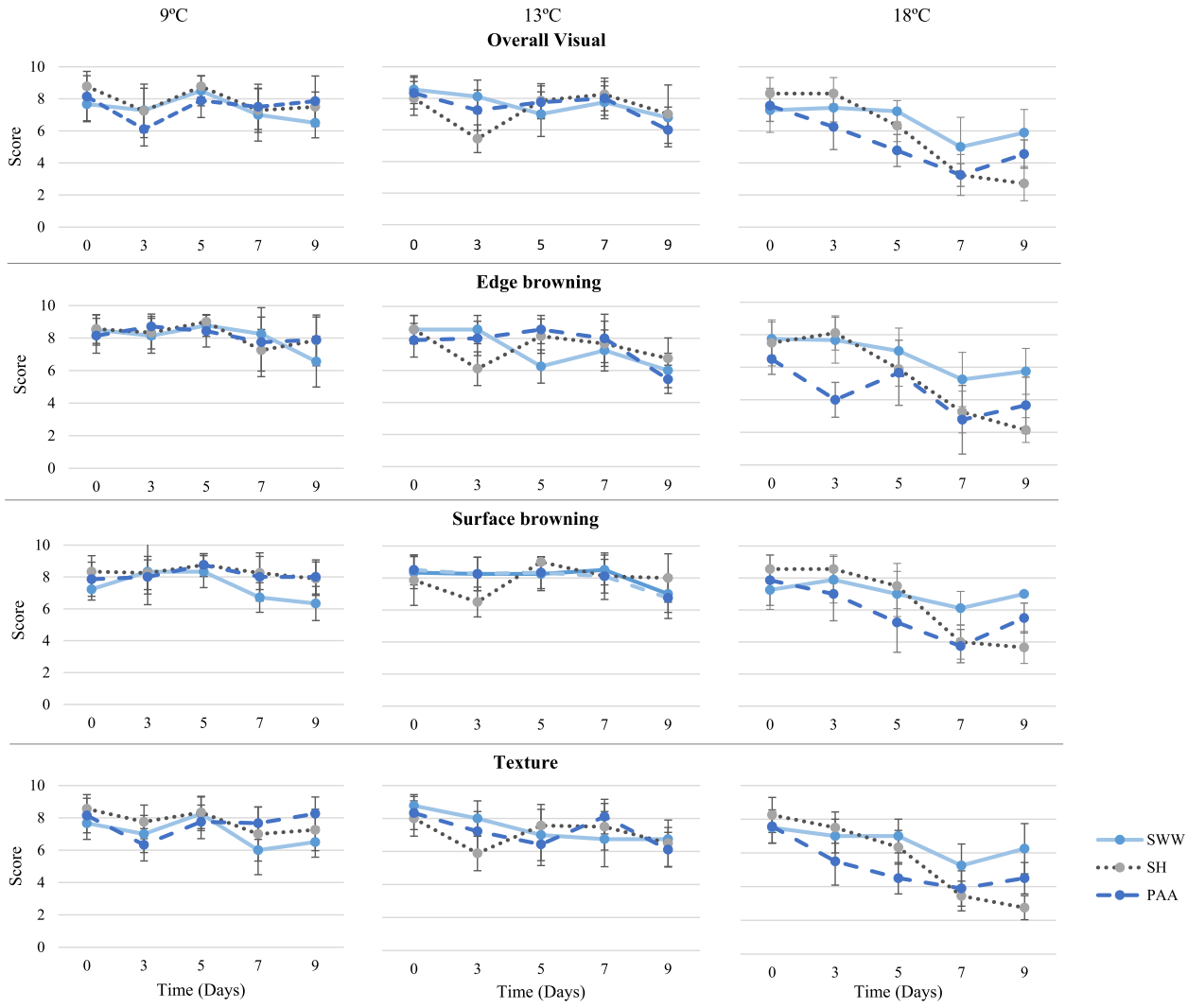

Fig. 3. Sensory quality evaluation of fresh-cut lettuce washed with Simulated wash water (SWW), SWW with 25 mg/L chlorine (SH) and SWW with 80 mg/L peroxyacetic acid (PAA), stored under modified atmosphere conditions at 9, 13, and 18 °C for 9 days.

Table 3
Initial and maximum populations of *Salmonella* observed in fresh-cut lettuce washed with Simulated Wash Water (SWW) and disinfectant solutions and stored under modified atmosphere conditions at 9, 13, and 18 °C for 9 days.

Treatment[1]	T (°C)[2]	N_0 (log CFU/g)[3]	N_{max} (log CFU/g)[4]
SWW	9	3.55 ± 0.34	5.44 ± 0.56[b5]
	13		6.31 ± 0.06[1,2]
	18		6.84 ± 0.41[1]
SH	9	3.24 ± 0.14	5.35 ± 0.71[2]
	13		6.60 ± 0.37[1]
	18		6.67 ± 0.23[1]
PAA	9	3.26 ± 0.14	4.63 ± 0.37[3]
	13		5.87 ± 0.32[2]
	18		7.15 ± 0.15[1]

[1] SWW: Simulated wash water, SH: SWW with 25 mg/L chlorine, PAA: SWW with 80 mg/L peroxyacetic acid.
[2] Storage temperature
[3] Initial population observed.
[4] Maximum population observed.
[5] Different letters in the same column indicate significant differences ($p < 0.05$) according to Tukey's test.

Table 3.

In all cases, *Salmonella* populations in the samples increased on the first day of storage and during the subsequent storage days (Fig. 4). Besides, none of the samples incubated at 9 °C was able to reach the maximum population density (*MPD*) during the 9-day storage period (Table 3 and Fig. 4). The values corresponded to 5.44 ± 0.56, 5.35 ± 0.71, and 4.63 ± 0.37 log CFU/g, which were statistically similar among treatments ($p = 0.2424$). Conversely, all samples stored at 13 °C and 18 °C reached the *MPD* for all treatments, represented by the final asymptotic concentration value. At 13 °C, the final values differed among treatments, corresponding to 6.60 ± 0.37 and 5.87 ± 0.32 log CFU/g for SH and PAA, respectively ($p < 0.05$). Therefore, samples subjected to PAA treatment reached lower concentrations than those observed for SH. At 18 °C, the maximum levels were statistically identical for both disinfectants ($p = 0.134$).

The growth potential (δ), calculated as the difference between N_f and N_0, indicated that, as expected, the highest δ was presented in samples stored at 18 °C. Samples treated with SWW, SH, and PAA showed δ values of 3.14 ± 0.54, 3.43 ± 0.35, and 3.89 ± 0.29 log CFU/g, respectively, which were statistically similar ($p = 0.1372$).

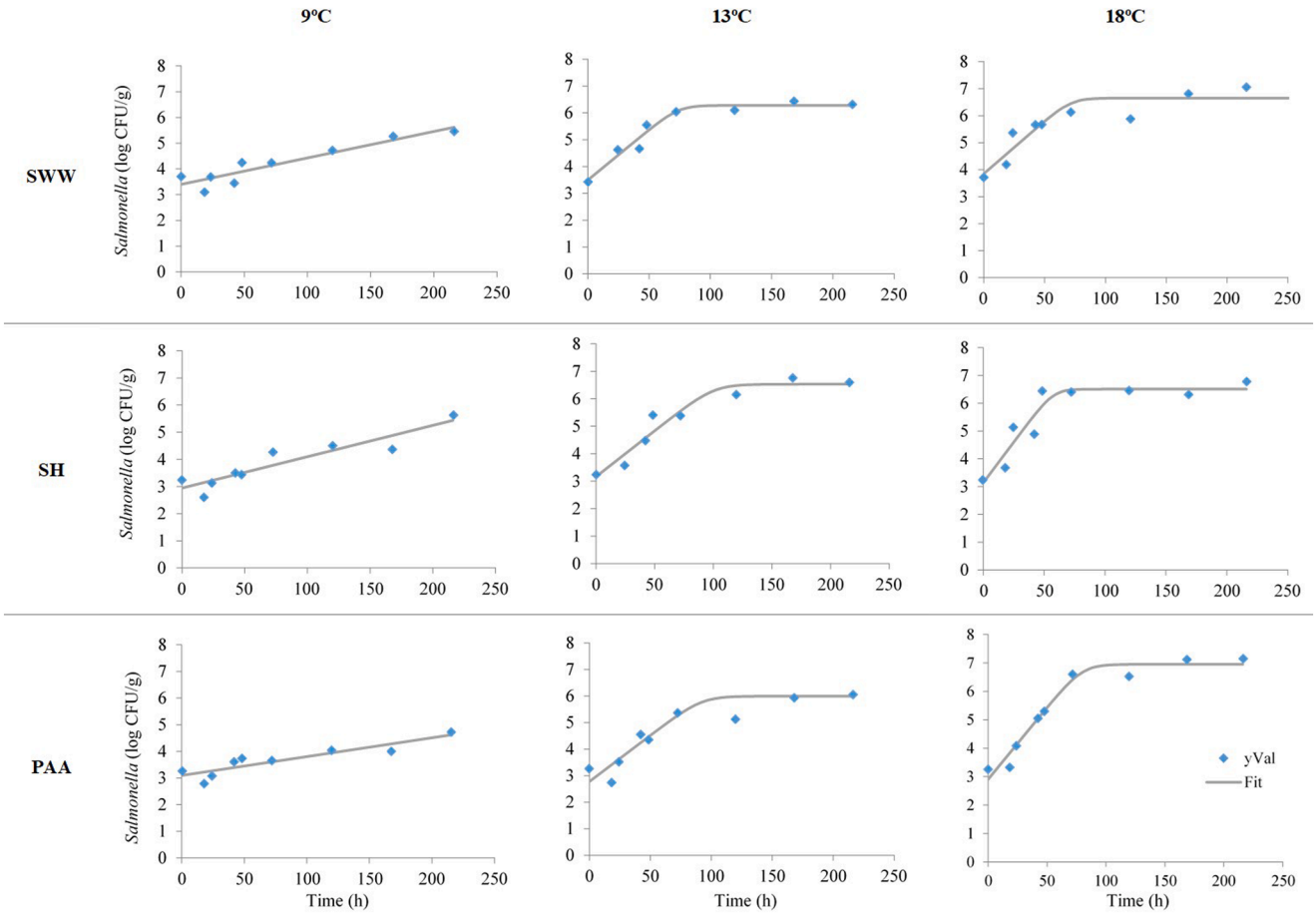

Fig. 4. Growth curves (points) and fit of the Baranyi & Roberts (1994) (solid line) growth primary model for *Salmonella* counts observed in fresh-cut lettuce washed with Simulated wash water (SWW), SWW with 25 mg/L chlorine (SH) and SWW with 80 mg/L peroxyacetic acid (PAA) stored under modified atmosphere conditions for 9 days at 9, 13, and 18 °C.

3.5. Influence of washing with water and different disinfectants on the estimated kinetic parameters of Salmonella in fresh-cut lettuce

Fig. 4 shows the fitting curves of the Baranyi & Roberts (1994) growth primary model derived from the *Salmonella* counts observed during 9 days of storage at the three different temperatures. The goodness-of-fit of the obtained models corresponded to $R^2 = 0.80–0.96$ and SE = 0.26–0.50.

Table 4 presents growth rates obtained from both the primary model and the selected secondary model. It is noteworthy that the model showed no lag time (λ) for all treatments (Fig. 4), including those using disinfectants, which is in agreement with other studies reporting that lag time was very short or absent. For instance, Ndraha et al. (2022) reported no lag time for all *Salmonella* strains at temperatures ranging from 10 °C to 25 °C. Similarly, in the study by Tarlak et al. (2020), *Salmonella* Reading showed no lag time in fresh-cut lettuce packaged in modified atmosphere and stored at 15 °C. In studies where lag time was observed, it was short and limited to around 24 h (Koseki & Isobe, 2005; Park, Yi Zhang, & Ha, 2019; Puerta-Gomez, Moreira, Kim, & Castell-Perez, 2013; Sant'Ana, Franco, & Schaffner, 2012; Yoon et al., 2014). For example, in the study by Park, Yi Zhang, & Ha (2019) , lettuce inoculated with *S.* Typhimurium and washed with chlorine (100 ppm) resulted in lag time values ranging from 25.86 to 30.46 h at 10 °C, 17.20 to 21.56 h at 15 °C, 8.13 to 12.57 h at 20 °C, and 2.45 to 6.70 h at 25 °C. In our study, since the first microbiological analysis was performed at 18 and 24 h, we cannot dismiss the possibility that *Salmonella* had a short lag time that was not reflected in the fitted model.

As can be seen from the values in Table 4, maximum growth rate increased with temperature. This fact about *Salmonella* growth in fresh-cut vegetables and fruit has been extensively reported in scientific literature (de Oliveira Elias, Noronha, & Tondo, 2018; Ma et al., 2016; Ndraha et al., 2022; Park, Yi Zhang, & Ha, 2019; Singh, Rahman, Sharma, & Yemmireddy, 2021; Tarlak et al., 2020).

Table 4
Growth primary and secondary growth parameters of *Salmonella* in fresh-cut lettuce washed with Simulated Wash Water (SWW) and disinfectant solutions stored under modified atmosphere conditions at different temperatures (9, 13, and 18 °C).

Treatments[1]	Primary growth Parameters			Secondary growth parameters[5]	
	μ_{max} (log CFU/h)[2]				
	9 °C	13 °C	18 °C	*b*	T_0
SWW	0.016 ± 0.003[3]	0.035 ± 0.002[1]	0.039 ± 0.009[1]	0.00237 ± 0.00065[2]	0.67 ± 3.58[2]
SH	0.011 ± 0.003[4]	0.034 ± 0.013[1]	0.054 ± 0.009[1]	0.00465 ± 0.00073[1]	6.2 ± 1.25[1]
PAA	0.010 ± 0.002[2]	0.042 ± 0.015[1]	0.048 ± 0.003[1]	0.00414 ± 0.00103[1]	5.2 ± 2.21[3]

[1] SWW: Simulated wash water, SH: SWW with 25 mg/L chlorine, PAA: SWW with 80 mg/L peroxyacetic acid.
[2] Maximum growth rate.
[3] Estimated parameter ± Standard error.
[4] Different letters in the same column indicate significant differences ($p < 0.05$) according to Duncan's test.
[5] *b* and T_o are regression parameters estimated from Bělehrádek-type models represented by Equation 1 (Bělehrádek,1926).

Statistical analysis of μ_{max} only showed significant differences between treatments at 9 °C, where its value was significantly lower in lettuce samples treated with SH and PAA compared to that obtained only for SWW ($p < 0.05$). The μ_{max} values decreased from 0.016 log CFU/h in water to 0.010 log CFU/h in PAA. In the work by Park, Yi Zhang, & Ha (2019), *Salmonella* growth in lettuce was also assessed with respect to two disinfectant treatments, reporting a decreased growth when a combination of high concentrations of chlorine and ultrasound was applied to samples compared to control treated with sterile distilled water. To the best of our knowledge, no other work has specifically analyzed the effect of disinfectants on *Salmonella* growth in lettuce or other leafy green produce.

The growth rate values obtained in our study were compared with literature data from lettuce and similar produces (Fig. 5). The graphical representation was made considering different temperature ranges to facilitate the comparison of data from similar or very close temperature values. At first sight, the kinetics of *Salmonella* exposed to disinfectants (this study and that of Park, Yi Zhang, & Ha, 2019) tended to be lower than those in which no sanitizing treatment was applied after *Salmonella* inoculation. These differences were especially evident for the 9–10 °C range (Fig. 5A) (de Oliveira Elias, Noronha, & Tondo, 2018; Koseki & Isobe, 2005; Ndraha, Goh, Tran, Chen, & Hsiao, 2022; Park, Yi Zhang, & Ha, 2019; Sant'Ana, Franco, & Schaffner, 2012; Veys, Elias, de, Sampers, & Tondo, 2016). However, this trend should be interpreted with caution, as it is limited to the presence of only two studies that include the effect of disinfectant on *Salmonella* growth, since most of the studies do not include a specific design to test this factor. Therefore, the growth rates from studies that did not consider disinfectant were the majority. It is particularly interesting that there was one group that showed much higher values than others. The studies by de Oliveira Elias et al. (2018), Sant'Ana et al. (2012) and Veys et al. (2016) reported values around 0.05 log CFU/h for 9–10 °C, which doubled those values registered in our study and other works (Koseki & Isobe, 2005; Ma et al., 2016; Ndraha et al., 2022; Puerta-Gomez et al., 2013; Park, Yi Zhang, & Ha, 2019). Due to the differences between studies, it is difficult to provide any explanation or cause. Studies may differ in temperature, strains, background microbiota, packaging conditions, and inoculum size, among other experimental factors. In addition, several studies have shown that growth kinetics could differ among pathogenic strains (Coleman et al., 2003; Lianou & Koutsoumanis, 2011; Sant'Ana et al., 2012).

The Bělehrádek-type models were used to describe growth rate dependence on temperature. However, the best performance according to SE was obtained for $m = 1$ and without transformation of μ_{max} ($R^2 > 0.83$, $SE < 0.03$). The notional temperature (T_0) usually differs from the actual minimum temperature (Ross & Dalgaard, 2004), although it can be considered an intrinsic property of the bacterial population (Ratkowsky, Lowry, McMeekin, Stokes, & Chandler, 1983). These values were in the range of the values reported in similar works on *Salmonella* and lettuce (Table 5) (Koseki & Isobe, 2005; Ndraha et al., 2022; Sant'Ana et al., 2012; Veys et al., 2016), and remain in line with the reported actual minimal growth temperature for these pathogens, which is around 4–5 °C (Food and Drug Administration, 2001), even though there are also studies reporting lower and negative values for T_0 (de Oliveira Elias et al., 2018; Xiao et al., 2021).

Specifically, in our work, T_0 values for the SH and SWW treatments were statistically different ($p < 0.05$), but they were significantly similar between SH and PAA ($p > 0.05$) (Table 4). Probably, the high prediction error, derived from the high variability inherent in a complex system such as lettuce, could hinder the analysis from reliably capturing potential differences. Despite the consequences of the high experimental variability, the apparent contrast observed between the T_0 values obtained from the disinfection treatments and SWW should not be overlooked. From a more conceptual perspective, different T_0 might reflect a different impact of the treatment on the pathogen kinetics, which could also be deduced from the statistical differences between treatments found for μ_{max} at 9 °C. A lower T_0 would indicate that microorganisms have a higher growth capacity at lower temperatures. The statistical differences ($p < 0.05$) found for the regression parameter b would be in line with this fact, suggesting a different kinetic profile of *Salmonella* in those samples treated with disinfectant.

Fig. 6 illustrates the fitted secondary models with the estimated regression parameters for each treatment. The observation of the entire temperature range may better reflect the impact of the washing treatment on *Salmonella* growth patterns. The model lines for *Salmonella* exposed to both disinfectants showed similar trends, overlapping over the temperature range tested and with a very similar slope. On the contrary, the model line from the kinetics of *Salmonella* exposed only to SWW presented a completely different pattern, showing a smaller intercept and slope and crossing the SH and PAA lines at around 12 °C (Fig. 6). The predicted kinetic behavior would suggest that, at temperatures below 12 °C, *Salmonell*a in fresh-cut lettuce treated with SWW exhibit a better growth capacity (i.e., μ_{max}) than *Salmonella* exposed to SH and PAA. Whereas at temperatures above 12 °C, the effect would be the opposite, with better *Salmonella* growth capacity in those samples treated with disinfectant (i.e., SH and PAA).

These results demonstrate that pre-growth stress, in terms of exposure to disinfectants, can determine *Salmonella* kinetics during storage. It is possible that, at lower temperatures, the presence of injured or stressed bacterial cells in lettuce samples treated with disinfectants (SH and PAA) could lead to a slower growth than uninjured *Salmonella* populations in lettuce washed only with water. In this regard, incubation temperature has been shown to have a significant impact on lesion repair (Liao & Fett, 2005). However, at higher temperatures, the better growth capacity of *Salmonell*a cells exposed to disinfectants cannot be explained by the same hypothesis. Some studies have reported that cells can be induced to a filamentous stage by chemical or environmental stress (pH, water activity, desiccation, etc.) (Giotis, Blair, & McDowell, 2007; Kieboom et al., 2006; Muhandiramlage, McWhorter, & Chousalkar, 2020). When these elongated cells are introduced under more favorable conditions, the filaments could divide and form numerous single cells rapidly, increasing the number of cells and, consequently, the population growth rate (Finn, Condell, McClure, Amézquita, & Fanning, 2013). Nonetheless, this is a hypothesis that needs to be specifically tested considering, in addition, the role of the endogenous microbiota, that, when reduced by disinfectants, could also facilitate pathogen growth. Investigations at molecular and microscopic level are also needed to gain a better understanding of the survival and growth mechanisms of *Salmonella* cells exposed to disinfectants during the washing step of fresh produce.

4. Conclusions

In this study, it was observed that the size of the *Salmonella* population in fresh-cut lettuce affected the removal capacity at the washing step, increasing when the population levels in the product were higher (≥5.5 log CFU/g). In addition, it was proven that peroxyacetic acid can be an effective alternative for the disinfection of *Salmonella* in fresh-cut lettuce, producing similar reduction levels to chlorine but with slightly better performance in reducing produce deterioration during storage.

The modeling approach based on the Bělehrádek-type models could satisfactorily describe *Salmonella* kinetics over the temperature range studied (9–18 °C). Interestingly, the close examination of the models and their parameters (T_0 and b) unveiled a remarkable impact of chlorine and peroxyacetic acid on *Salmonella* kinetics during storage, reducing the growth rate at lower temperatures (≤12 °C) but increasing it at higher temperatures compared to fresh-cut lettuce treated only with water. Further research will be needed to better understand the physiological and biological mechanisms caused by the disinfectant responsible for the observed kinetic behavior.

The data and models developed in this study could be useful inputs to risk assessment studies applied to fresh-cut produce, providing more

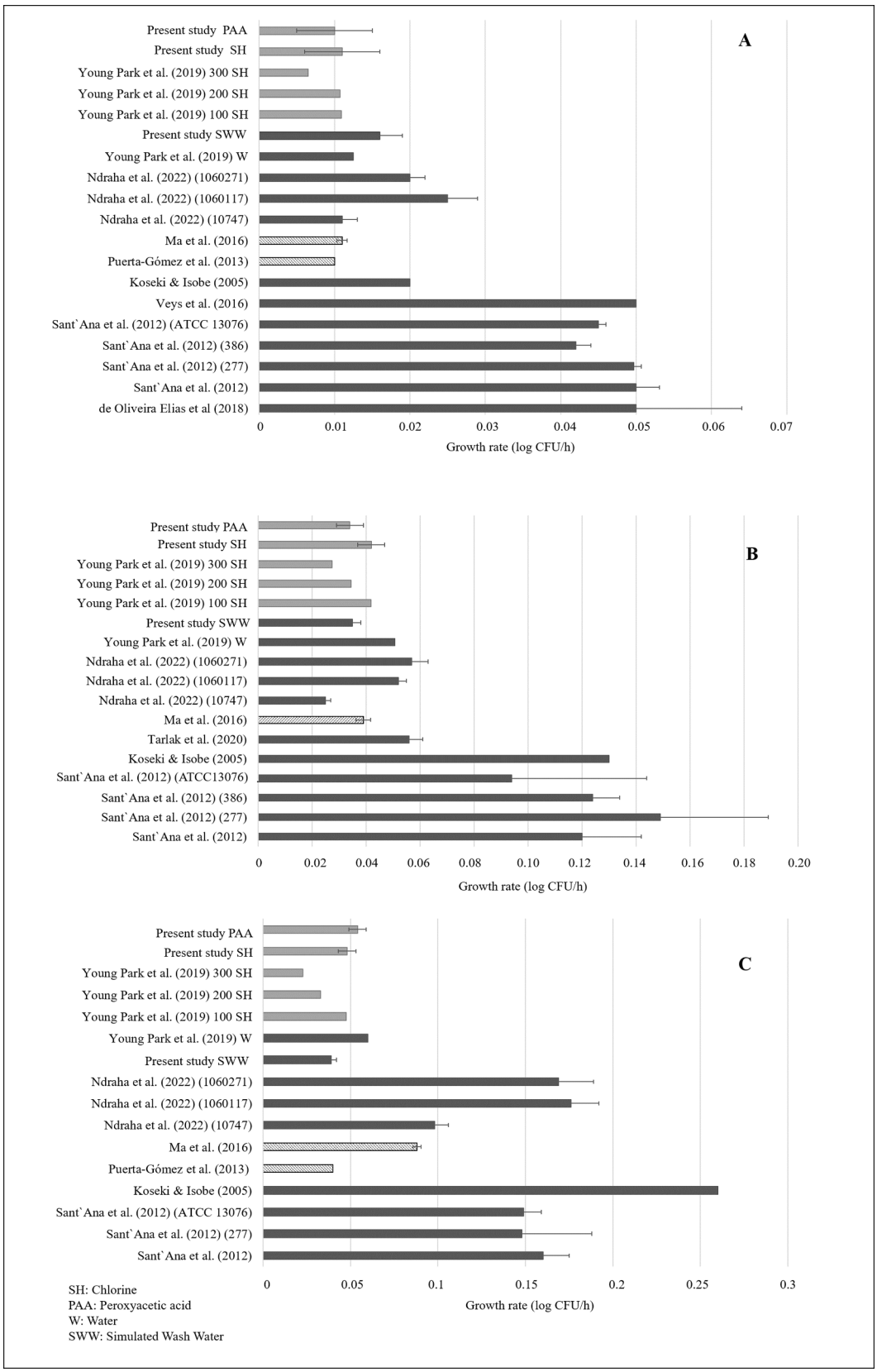

Fig. 5. Bar plots representing maximum growth rates (log CFU/h) of *Salmonella* strains in lettuce (solid color) and other produces (stripped color) reported by different studies and our study at 9–10 °C (A), 13–15 °C (B), and 18–20 °C (C). Bars with clear grey color define studies in which a disinfectant treatment was applied after *Salmonella* inoculation, while dark grey color stands for those without any disinfectant treatment after pathogen inoculation. The value between parentheses refers to the *Salmonella* strain used in the study, while for those studies applying a disinfection treatment, the value shown before the type of disinfectant (i.e., PAA or SH) indicates the concentration level used.

Table 5
Parameters of the root-square model (b and T_0) for growth rate of *Salmonella* in lettuce sample at different storage temperatures.

Treatment[1]	Temperature range (oC)	b	T_0	Reference
SWW	9–18	0.0024 ± 0.0006	0.67 ± 3.59	Present study
SH	9–18	0.0047 ± 0.0007	6.25 ± 1.26	Present study
PAA	9–18	0.0041 ± 0.0010	5.24 ± 2.21	Present study
Untreated	10–25	0.028	8.805	Ndraha et al. (2022)
Untreated	10–25	0.028	5.466	Ndraha et al. (2022)
Untreated	10–25	0.027	5.477	Ndraha et al. (2022)
Untreated	10–25	0.033	4.96	Koseki and Isobe (2005)
Washed/tap water	7–30	0.0178	6.65	Sant'Ana et al. (2012)
Untreated	5–37	0.027	5.42	Veys et al. (2016)
Untreated	5–25	0.0339	1.92	de Oliveira Elias et al. (2018)
Untreated	7–30	0.018	−7.6	Xiao et al. (2021)
Untreated	7–30	0.016	−13.5	Xiao et al. (2021)

[1] SWW: Simulated wash water, SH: SWW with 25 mg/L chlorine, PAA: SWW with 80 mg/L peroxyacetic acid.

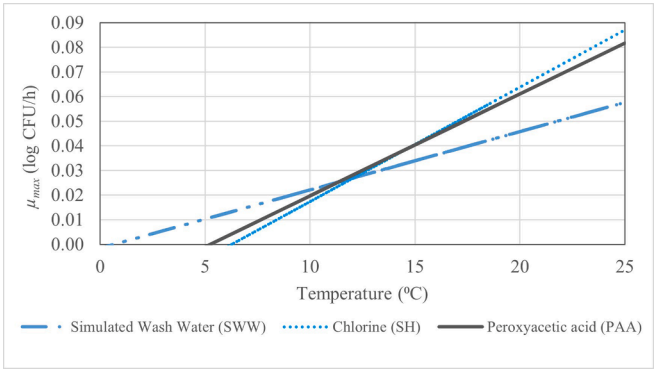

Fig. 6. Fitted secondary models describing growth rate dependence on temperature of *Salmonella* in fresh-cut lettuce subjected to disinfection and washing treatments and stored at different temperatures (9–18 °C) under modified atmosphere.

complete and accurate risk estimates.

CRediT authorship contribution statement

Sofia Griselda Cuggino: Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Guiomar Posada-Izquierdo:** Resources, Methodology, Writing – review & editing. **Isabel Bascón Villegas:** Methodology. **Martin Gustavo Theumer:** Conceptualization, Methodology, Formal analysis, Supervision, Writing – review & editing. **Fernando Pérez-Rodríguez:** Resources, Conceptualization, Methodology, Formal analysis, Supervision, Writing – review & editing, Project administration, Funding acquisition.

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.

Acknowledgements

This work has been developed as part of the project BioFreshCloud, with reference PRIMA-S2-2019-PCI2020-112015, which is part of the PRIMA programme supported by the European Union and funded by MCIN/AEI/10.13039/501100011033 and European Union "NextGenerationEU/PRTR". Sofia Cuggino is holder of a doctoral scholarship of the Asociación Universitario Iberoamericana de Posgrado (AUIP). Martín Gustavo Theumer is a career investigator from the National Research Council from Argentina (CONICET).

We thank Ms. Silvina A. Colla, Sworn Translator of English, for the linguistic revision of the manuscript.

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