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Modeling the growth rate and lag time of different strains of *Salmonella enterica* and *Listeria monocytogenes* in ready-to-eat lettuce

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ABSTRACT

The growth parameters (growth rate, μ and lag time, λ) of three different strains each of *Salmonella enterica* and *Listeria monocytogenes* in minimally processed lettuce (MPL) and their changes as a function of temperature were modeled. MPL were packed under modified atmosphere (5% O_2, 15% CO_2 and 80% N_2), stored at 7–30 °C and samples collected at different time intervals were enumerated for *S. enterica* and *L. monocytogenes*. Growth curves and equations describing the relationship between μ and λ as a function of temperature were constructed using the DMFit Excel add-in and through linear regression, respectively. The predicted growth parameters for the pathogens observed in this study were compared to ComBase, Pathogen modeling program (PMP) and data from the literature. High R^2 values (0.97 and 0.93) were observed for average growth curves of different strains of pathogens grown on MPL. Secondary models of μ and λ for both pathogens followed a linear trend with high R^2 values (>0.90). Root mean square error (RMSE) showed that the models obtained are accurate and suitable for modeling the growth of *S. enterica* and *L. monocytogenes* in MP lettuce. The current study provides growth models for these foodborne pathogens that can be used in microbial risk assessment.

1. Introduction

Ready-to-eat minimally processed vegetables (MPV) have gained more importance in the last 20 years due to consumers' demand for fresh, convenient, preservative-free foods that may promote health (Zink, 1997; Jacxsens et al., 2010). In Brazil, the MPV market increases yearly, with lettuce representing the most consumed leafy vegetable in the country (Sato et al., 2007).

Concurrent with the increase in their production and consumption, ready-to-eat vegetables have been associated with progressively more disease outbreaks (Harris et al., 2003; Sivapalasingam et al., 2004; Little and Gillespie, 2008; Lynch et al., 2009). Between 1999 and 2008, 6062 foodborne disease outbreaks were reported in Brazil, of which 114 involved vegetables (Anonymous, 2010). Moreover, several surveys have been carried out to evaluate the microbiological quality of MPV in Brazil (Fröder et al., 2007; Silva et al., 2007; Oliveira et al., 2011) and abroad (Abadias et al., 2008; Ailes et al., 2008; Meldrum et al., 2009; Caponigro et al., 2010; Giusti et al., 2010) and have reported on the prevalence and/or countable levels of pathogens such as *Salmonella* and *Listeria monocytogenes* in these products (Fröder et al., 2007; Little et al., 2007; Abadias et al., 2008; Cordano and Jacquet, 2009; Giusti et al., 2010; Oliveira et al., 2010; Quiroz-Santiago et al., 2009; Sant'Ana et al., 2011).

Salmonella is the major bacterial challenge for the safety of a wide variety of foods around the world (Greig and Ravel, 2009), and it is the main etiological agent of outbreaks in which fresh produce items are implicated (Tauxe, 1997; Harris et al., 2003; Little and Gillespie, 2008). In Brazil, salmonellae are responsible for approximately 47% of the notified foodborne disease outbreaks (Anonymous, 2010). The primary source of *Salmonella* appears to be animal feces, while contaminated soil and water used for irrigation may also contribute to its spread to vegetables (Krtinić et al., 2010).

L. monocytogenes is a ubiquitous foodborne pathogen with a known ability to grow under refrigeration temperatures (Hanning et al., 2008) and to survive and adapt to adverse environments (Gandhi and Chikindas, 2007). Although it causes fewer foodborne diseases outbreaks than *Salmonella* (Greig and Ravel, 2009), *L. monocytogenes* represents a major food safety concern due to the high mortality rate of those sickened with listeriosis (Bennion et al., 2008).

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In spite of the importance of estimating microbial growth kinetics in the context of quantitative risk assessment (QRA), simple addition of modeling data into QRA models might not be enough for obtaining more accurate models. Several studies have shown substantial differences in the growth kinetics among different strains of foodborne pathogens (Guillier and Augustin, 2006; Lindqvist, 2006; Koutsoumanis, 2008; Pal et al., 2008; Valík et al., 2008; Augustin et al., 2011; Lianou and Koutsoumanis, 2011). It is recognized that intra-species variability of microbial growth kinetics may influence the accuracy of outputs from risk assessment models (Delignette-Muller and Rosso, 2000). Therefore, information on the variability of growth kinetics among different strains of foodborne pathogens and how these parameters are affected by variations in the environment should be developed to help obtain reliable outputs from risk assessment (Lianou and Koutsoumanis, 2011). That is why, in this study, the growth of three different strains of *Salmonella enterica* and *L. monocytogenes* in minimally processed lettuce as a function of temperature (7–30 °C) were modeled separately using both primary and secondary models.

2. Material and methods

2.1. Strains and preparation of cell suspensions

Three different strains of *S. enterica* and *L. monocytogenes* were used in this study. *S. enterica* strains were from serotype Typhimurium (# 277 and 386) and Enteritidis ATCC 13076 while the *L. monocytogenes* strains were of serotype 4b (#: 413, 494 and 581). Except for the *Salmonella* ATCC strain, the other two strains used in this study were isolated from MPV marketed in Sao Paulo, Brazil.

Each strain of *S. enterica* and *L. monocytogenes* was grown separately in 10 mL of Tryptic Soy Broth (Oxoid, Basingstoke, UK) (TSB) and TSB supplemented with 0.6% yeast extract (Oxoid, Basingstoke, UK) (TSB-YE), respectively. Broth tubes were incubated at 37 °C/24 h, following two successive inoculations. The third inoculation was carried in 90 mL of the culture medium, following centrifugation at 2810*g* at 4 °C for 10 min (Mikro 22R, Hettich Zentrifugen, Germany) for removing debris and residual culture media. After centrifugation, the supernatant was discarded and pellets were washed with sterile 0.1% peptone water. Centrifugation and washing procedures were repeated three times and cells were re-suspended in sterile 0.1% peptone water. Final cell concentrations were adjusted at optical density 0.5 (OD_{630}) (10^8 CFU/mL) using an Ultrospec 200 UV/visible spectrophotometer (Pharmacia Biotech, Buckinghamshire, UK). Each suspension was separately prepared just before the experiments.

2.2. Minimal processing of lettuce and its inoculation with pathogens

The two most consumed varieties of lettuce in Brazil (iceberg – *Lactuca sativa* var. *capitata* and crisp – *L. sativa* var. *crispa*) were purchased from produce outlets located in the city of Sao Paulo, Brazil, and transported to the lab within 1 h. The external, damaged leaves and core were discarded before washing. The leaves were individually washed in tap water and were cut into strips of approximately 2 cm^2 width with a disinfected knife. The two varieties of lettuce were mixed in the same proportion and shredded leaves were immersed into a disinfected 20 L plastic basin containing Sumaveg solution (JohnsonDiversey, Sao Paulo, Brazil) at 100 ppm for 15 min. Chlorine levels were checked using pH paper test strips (Hydrion, Micro Chlorine, Johnson Wax Professional, Sturtevant, USA). Shredded lettuce leaves were then transferred into a previously disinfected plastic basin containing sterile distilled water, rinsed and allowed to dry in a laminar flow cabinet for at least 30 min.

Sterile distilled water (4 L) in pails lined with sterile plastic bags was inoculated with each suspension to reach a final concentration of 10^1–10^2 CFU/g of each pathogen strain. Shredded lettuce leaves were inoculated by dipping into solutions containing different strains of *S. enterica* and *L. monocytogenes* for 15 min. Removal of excess water was done with the aid of a previously sanitized salad spinner (Model DD1056Y, Casa da Moda, Brazil) in a biosafety cabinet. Control samples were prepared by repeating the same procedure above in non-inoculated sterile distilled water.

2.3. Packaging, storage of minimally processed lettuce and enumeration of pathogens

Twenty-five grams of contaminated, minimally processed lettuce was packaged in bags made of multilayer films (external layer: bi-oriented polypropylene; internal layer: blend or co-extruded structure of low-density polyethylene with linear low-density polyethylene) under modified atmosphere (5% O_2, 15% CO_2 and 80% N_2 – White Martins, Osasco, Brazil) using vacuum-sealing machine AP 500 (Tecmaq, Sao Paulo, Brazil). This plastic material has been used by several fresh vegetable processing facilities in Brazil and has 62 μm thickness, O_2 permeability of 1.375 $m^3\ m^{-2}\ day^{-1}$ at 23 °C and water steam permeability of 3.5 g water $m^{-2}\ day^{-1}$ at 38 °C and 90% relative humidity. Bags of lettuce were stored at 7, 10, 15, 20, 25 and 30 °C, and samples were collected at different time intervals and were analyzed for *S. enterica* or *L. monocytogenes*. The time intervals between sampling varied depending on the temperature of storage and ended once the microorganisms reached stationary phase.

S. enterica was enumerated by stomaching the vegetables (25 g) and the diluents, following pour-plate of 1 mL of serial dilutions prepared in 0.1% sterile peptone water in mannitol lysine crystal violet brilliant green (MLCB) agar (Oxoid, Basingstoke, UK). MLCB plates were further incubated at 37 °C for 24h. *L. monocytogenes* was enumerated in the same approach, following pour-plate of 1 mL of serial dilutions prepared in 0.1% sterile peptone water in Oxford agar (Oxoid, Basingstoke, UK) with incubation at 37 °C for 48 h. Approximately 3–5 colonies per point along the growth curve were selected for further confirmation by polyvalent serotyping (*S. enterica*) or biochemical tests as described in ISO 11290-2 (*L. monocytogenes*) (Anonymous, 1998). The limit of enumeration of the method used was 10^1 CFU/g. Experiments were replicated twice for each strain and temperature studied. Results were expressed as CFU/g and data were used for primary predictive modeling.

2.4. Modeling growth parameters of three different strains of S. enterica and L. monocytogenes in minimally processed lettuce

Growth curves for each temperature and strain were built separately by fitting data to the Baranyi model (Baranyi and Roberts, 1994) (Equations (1)–(3)) using DMFit version 2.1 Excel® add-in (www.ifr.ac.uk/safety/DMfit).

$$\ln(N(t)) = \ln(N_0) + \mu_{max}A(t) - \ln\left[1 + \frac{e^{\mu_{max}A(t)} - 1}{e^{(N_{max} - N_0)}}\right] \quad (1)$$

$$A(t) = t + \frac{1}{\mu_{max}}\ln\left(\frac{e^{(-\mu_{max}t)} + q_0}{1 + q_0}\right) \quad (2)$$

$$\lambda = \frac{\ln\left(1+\frac{1}{q_0}\right)}{\mu_{max}} \quad (3)$$

Where: $\ln(N(t))$ = log of cell concentration at time t [h] (CFU/g); $\ln(N_0)$ = log of initial cell concentration (CFU/g); μ_{max} = exponential growth rate (log CFU/g/h); $\ln(N_{max})$ = log of maximum cell concentration; q_0 [-] = parameter expressing the physiological state of cells when $t = t_0$; λ = lag time (h).

The Ratkowsky model (Ratkowsky et al., 1982) was used to describe μ and λ as a function of storage temperature (Equation (4)):

$$\sqrt{r} = b(T - T_0) \quad (4)$$

In this model, $\sqrt{r}$ is the square root of maximum growth rate, b is the slope of the regression line, T is temperature and T_0 is a conceptual minimum temperature for microbial growth, where T was given in °C. For λ, transformation of data to natural logarithm (Ln) was needed to describe its relationship with storage temperature.

2.5. Model evaluation

Computer programs (ComBase Predictor [http://modelling.combase.cc/] and the Pathogen Modeling Program 7.0 – PMP [http://pmp.arserrc.gov/PMPOnline.aspxi]) were used to estimate μ and λ for further comparison with the data obtained in this study. The PMP and ComBase models were run with the following selected parameters: pH = 6.8, initial level of log 3 CFU/mL of the pathogen, and a_w = 0.995 (equal to 0.9 g/dL of NaCl). The pH and a_w values were chosen based on previous measurements carried out in our laboratory as described by Scott et al. (2001). PMP models used were "*Salmonella* aerobic growth" and "*L. monocytogenes* anaerobic growth" while ComBase models used were "salmonellae with CO_2 (%)" and "*L. monocytogenes* with CO_2 (%)". The aerobic growth model for *Salmonella* was chosen because PMP does not include anaerobic or modified atmosphere growth models for this pathogen. In the case of the ComBase models, CO_2 was set at 15%, *i.e.*, the initial % of CO_2 used in modified atmosphere. The minimal temperature used for predictions for the PMP *Salmonella* models were 10 °C as these models did not allow selection of a lower temperature. Additionally, our data were compared with the μ and λ reported in selected literature (Koseki and Isobe, 2005a,b), here referred to as "Koseki-derived model." Procedures previously adopted to transform data obtained in this study for secondary modeling were also used to deal with the data obtained from literature and from ComBase and PMP programs.

2.6. Statistical analysis

The μ and λ of the different strains of *Salmonella* and *L. monocytogenes* were checked for significant statistical differences ($p \leq 0.05$), employing one-factor analysis of variance (ANOVA) followed by Duncan's test. Growth parameters for both pathogens were also compared to each other. Statistical analyses were carried out in Assistat version 7.5 free software (Campina Grande, Brazil) (Silva and Azevedo, 2002). In addition, fitting of the models was evaluated by the coefficient of determination (R^2) and their performance was checked by the root mean square error (RMSE).

3. Results

S. enterica and *L. monocytogenes* survived, but did not grow on minimally processed lettuces stored at 5 °C. Growth curves started with an initial population of 10^1–10^2 CFU/g and final populations of *S. enterica* and *L. monocytogenes* varied between 10^5–10^6 CFU/g and 10^4–10^7 CFU/g, respectively. The average R^2 values for growth curves of the three different strains of *S. enterica* and *L. monocytogenes* inoculated on minimally processed lettuce leaves and fitted to Baranyi model were 0.97 and 0.93, respectively (figures not shown). Control samples did not show growth of *S. enterica* or *L. monocytogenes* during storage at different temperatures and periods.

Table 1 shows the average growth kinetic parameters for each of the three different strains of *S. enterica* and *L. monocytogenes* on packed minimally processed lettuce. At 7 and 10 °C *S. enterica* presented lower λ in comparison to *L. monocytogenes* ($p < 0.05$). With the rise in temperature, λ did not differ among the pathogens ($p > 0.05$) except at 20 °C. *S. enterica* grew faster than *L. monocytogenes* over the temperature range ($p < 0.05$), except at 20 °C. When growth parameters among strains of the same

Table 1
Growth kinetic parameters (μ, log CFU/g/h and λ, h) of three different strains of *S. enterica* and *L. monocytogenes* on packed minimally processed lettuce stored at 7–30°C[a,b].

Pathogen	Growth parameters	Strains	Temperature (°C)					
			7	10	15	20	25	30
Salmonella spp.	μ	277	0.053 ± 0.010[a]	0.0497 ± 0.001[a]	0.149 ± 0.04[a]	0.148 ± 0.04[a]	0.328 ± 0.002[a]	0.44 ± 0.09[a]
		386	0.061 ± 0.008[a]	0.042 ± 0.002[b]	0.124 ± 0.01[a]	0.138 ± 0.16[a]	0.300 ± 0.007[a]	0.32 ± 0.03[a]
		ATCC	0.0635 ± 0.006[a]	0.045 ± 0.001[a,b]	0.094 ± 0.05[a]	0.149 ± 0.01[a]	0.315 ± 0.001[a]	0.36 ± 0.03[a]
		Average	0.06 ± 0.0045[A]	0.05 ± 0.0031[A]	0.12 ± 0.022[A]	0.16 ± 0.015[A]	0.31 ± 0.011[A]	0.37 ± 0.05[A]
L. monocytogenes	μ	413	0.0165 ± 0.003[a]	0.0225 ± 0.006[a]	0.0656 ± 0.04[a]	0.172 ± 0.02[a]	0.152 ± 0.003[a]	0.24 ± 0.01[a]
		494	0.0191 ± 0.008[a]	0.0272 ± 0.009[a]	0.0605 ± 0.05[a]	0.069 ± 0.01[b]	0.122 ± 0.001[a]	0.20 ± 0.02[a]
		581	0.0141 ± 0.001[a]	0.0233 ± 0.010[a]	0.0495 ± 0.01[a]	0.094 ± 0.01[b]	0.110 ± 0.002[a]	0.19 ± 0.01[a]
		Average	0.02 ± 0.002[B]	0.02 ± 0.002[B]	0.06 ± 0.007[B]	0.11 ± 0.04[A]	0.13 ± 0.017[B]	0.22 ± 0.02[B]
Salmonella spp.	λ	277	25.2 ± 1.2[a]	12.8 ± 2.3[a]	4.7 ± 2.1[a]	4.4 ± 0.1[a,b]	1.7 ± 0.4[a]	1.5 ± 0.2[a]
		386	26.5 ± 1.6[a]	11.8 ± 3.1[a]	7.4 ± 2.0[a]	5.4 ± 0.6[a]	2.2 ± 0.7[a]	2.4 ± 1.1[a]
		ATCC	28.4 ± 2.1[a]	14.3 ± 0.9[a]	5.4 ± 0.9[a]	3.8 ± 0.5[b]	1.8 ± 0.1[a]	1.1 ± 0.4[a]
		Average	26.7 ± 1.3[B]	12.9 ± 1.0[B]	5.9 ± 1.1[A]	4.5 ± 0.7[A]	1.9 ± 0.2[B]	1.6 ± 0.6[A]
L. monocytogenes	λ	413	45.5 ± 11.4[a]	39.1 ± 20.1[a]	8.4 ± 7.7[a]	6.9 ± 2.6[a]	4.3 ± 0.5[a]	1.4 ± 0.4[b]
		494	56.6 ± 13.7[a]	44.6 ± 1.8[a]	14.4 ± 4.4[a]	5.4 ± 0.9[a]	3.6 ± 0.3[a]	2.5 ± 0.3[a,b]
		581	76.2 ± 10.0[a]	48.7 ± 8.2[a]	8.4 ± 1.5[a]	5.7 ± 1.4[a]	3.2 ± 0.1[a]	3.4 ± 0.7[a]
		Average	59.4 ± 12.7[A]	44.1 ± 3.9[A]	10.4 ± 2.8[A]	5.9 ± 0.6[A]	3.7 ± 0.5[A]	2.4 ± 0.8[A]

[a] Different superscript letters in the same column indicate significant differences ($p < 0.05$) for growth rate or lag time of different strains of the same pathogen according to Duncan's test.
[b] Different superscript capital letters in the same row indicate significant differences ($p < 0.05$) for average values of growth rate or lag time between *Salmonella enterica* and *L. monocytogenes* according to Duncan's test.

pathogen were evaluated, significant differences were observed for *S. enterica* only at 10 °C (growth rate) and 20 °C (lag time) ($p < 0.05$). The highest variations in μ and λ in salmonellae were observed when the growth took place at 30 °C. The μ varied between 0.32 log CFU/g/h and 0.44 log CFU/g/h in *S.* Typhimurium strains (#277 and 386). In addition, λ values varied from 1.1 h for *S.* Enteritidis ATCC 13076–2.4 h for *S.* Typhimurium (#386) (Table 1). It was found that *S.* Enteritidis ATCC 13076 presented slightly higher λ in comparison to strains 277 and 386 at 7 and 10 °C, while the strain 386 (*S.* Typhimurium) presented higher λ in the range of 15–30 °C. *S.* Typhimurium 277 presented an overall higher μ that was more evident at 25–30 °C.

Significant differences were observed at 20 °C (μ) and 30 °C (λ) ($p < 0.05$) for *L. monocytogenes* (Table 1). *L. monocytogenes* strain 581 presented slightly higher lag time at 7–10 °C, while strain 413 presented higher μ between 15 and 30 °C.

The relationship between the storage temperature of minimally processed lettuce and the average growth parameters (μ and λ) for each strain of *S. enterica* and *L. monocytogenes* is shown in Fig. 1. The relationship between the growth parameters and temperature for both pathogens followed a linear trend with high R^2 values (>0.90). Certain data points were out of the 95% confidence intervals, although the mean values are within the 95% CI (Fig. 1).

Fig. 2 presents a comparison of linear regression among the average growth parameters of *S. enterica* and *L. monocytogenes* obtained in this study, PMP, ComBase and Koseki. The model for λ of *S. enterica* (Fig. 2A) derived from Koseki was very close to the model obtained in this study, mainly at the points of 15, 20 and 25 °C. In this case, the ComBase and PMP-derived models presented higher slopes. On the other hand, secondary models for *L. monocytogenes* derived from ComBase, PMP and Koseki followed a similar pattern, with most of the data falling within the 95% CI of average data obtained in this study (Fig. 2-B). Models for μ of *S. enterica* (Fig. 2-C) derived from ComBase and PMP showed the greatest slope, followed by the model derived from Koseki. The secondary model for μ of *S. enterica* obtained in this study did not follow the same trend as the three other models. Conversely, the secondary model for *L. monocytogenes* derived from Koseki followed the same trend of the model derived from data obtained in this study, while models derived from PMP and ComBase presented the greatest slope (Fig. 2-D).

Equations describing the relationship of growth parameters and temperature for *S. enterica* and *L. monocytogenes* grown in packed minimally processed lettuce from 7 to 30 °C are shown in Equations (5)–(6) and (7)–(8), respectively:

$$\sqrt{\mu} = 0.0178(T - 6.65) \quad (5)$$

$$\ln(\lambda) = -0.118(T + 34.84) \quad (6)$$

$$\sqrt{\mu} = 0.0144(T - 1.96) \quad (7)$$

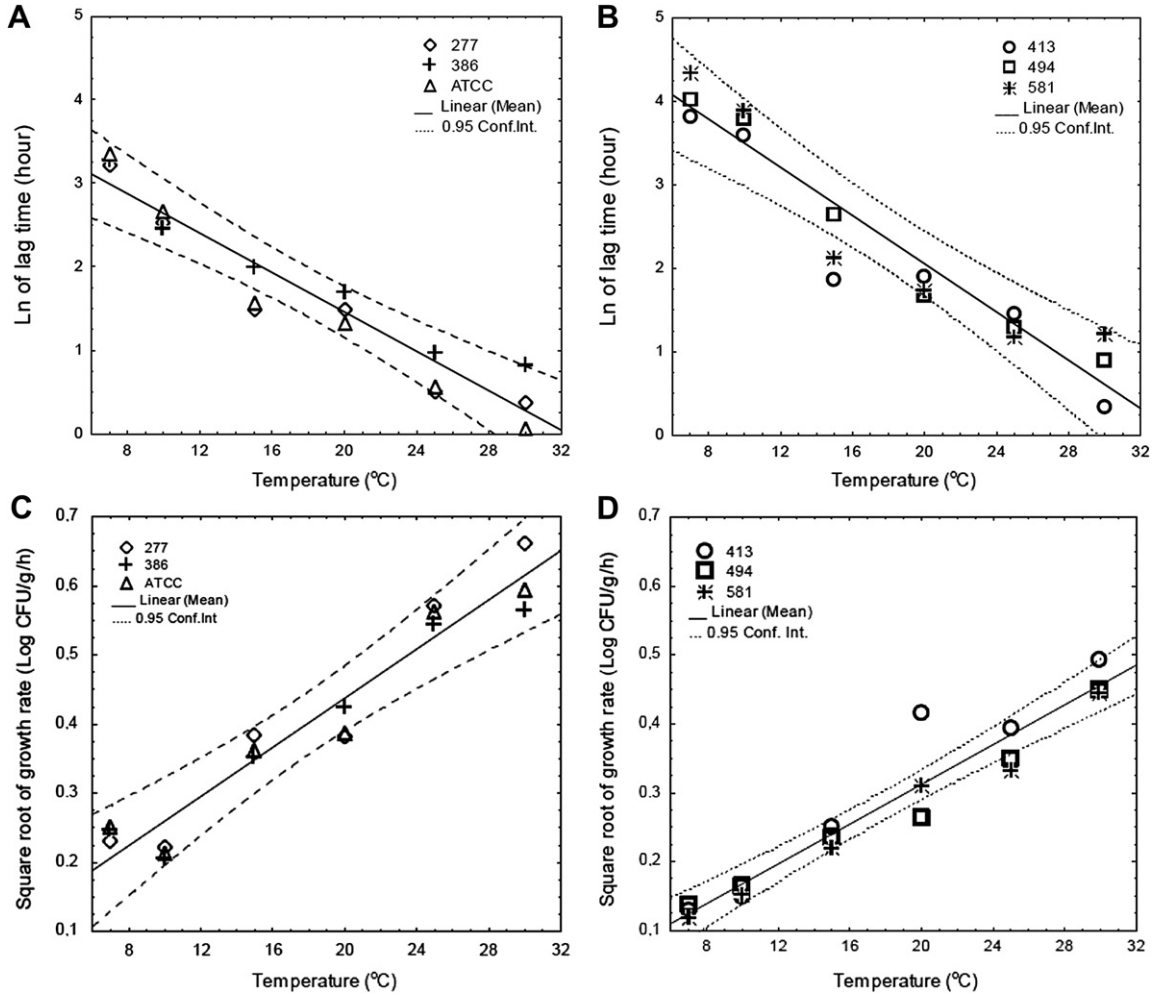

Fig. 1. Relationship between average growth parameters (μ and λ) of *Salmonella enterica* (A and C) and *L. monocytogenes* (B and D) strains and temperature of storage of minimally processed lettuce.

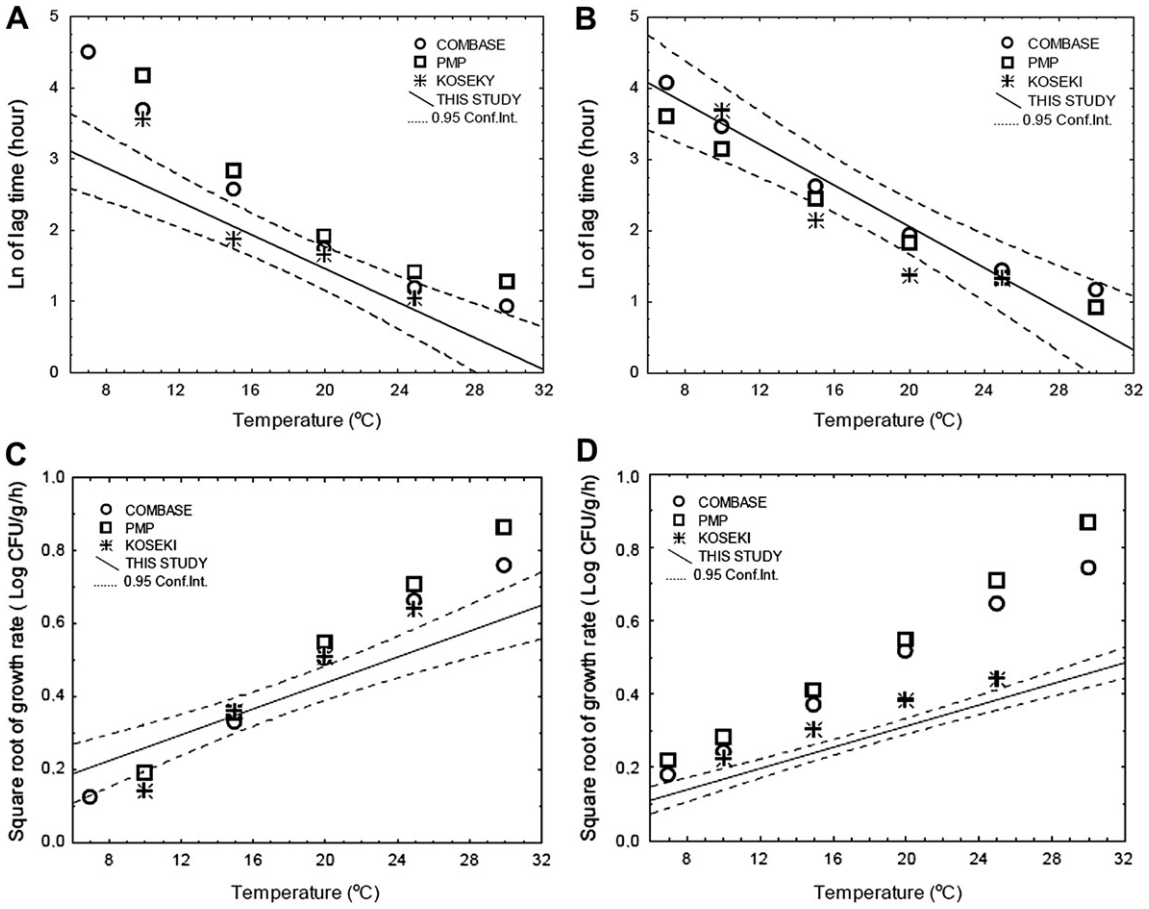

Fig. 2. Comparison of the linear regression models for (μ and λ) derived from ComBase, PMP, Koseki (symbols) and average data obtained in this study (solid lines).

$$\ln(\lambda) = -0.1445(T + 35.3) \quad (8)$$

RMSE values obtained for μ and λ models of *S. enterica* were 0.033 and 0.214, respectively. RMSE values for *L. monocytogenes* models were 0.015 (μ) and 0.271 (λ), respectively. It can be noticed that RMSE values for μ models were lower than for λ, no matter the pathogen.

4. Discussion

Despite the increasing association of vegetables with foodborne disease outbreaks in recent years (Lynch et al., 2009), few studies have focused on predicting the growth of pathogens in fresh vegetables (Koseki and Isobe, 2005a,b; Crépet et al., 2009; Ding et al., 2010) when compared to meat and dairy products (McMeekin, 2007; Poirazi et al., 2007; Vissers et al., 2007; Oscar, 2009; Cornu et al., 2010; Limbo et al., 2010; McMeekin et al., 2010; Schvartzman et al., 2010; Lindqvist and Lindblad, 2011). Therefore, there is a need for more data on the growth of foodborne pathogens in these products.

Studies on the growth modeling of pathogens are built with a higher starting initial population (*i.e.*, $>10^3$–10^4 CFU/g) (Koseki and Isobe, 2005a,b; Ding et al., 2010) of pathogens than that of naturally-contaminated foods, such as fresh vegetables (*i.e.*, $<10^1$–10^2 CFU/g) (Sagoo et al., 2003; Little et al., 2007; Cordano and Jacquet, 2009; Oliveira et al., 2010; Sant'Ana et al., 2011). However, predictive models should match the complexity of foods of concern, including low pathogens contamination levels, in order to provide more realistic outputs (Mejlholm et al., 2010). Therefore, herein the growth kinetic parameters (μ and λ) of three different strains of *S. enterica* and *L. monocytogenes* on packed MPV were predicted with low initial starting populations (10^1–10^2 CFU/g). The average growth curves of three different strains of these pathogens presented higher R^2 values (>0.93) than those found in the separate curves for each strain (0.83 and 0.90). R^2 is defined as the proportion of the variability in the data set that is explained by the statistical model (Myers et al., 2009). Therefore, the improvement in curve fitting for the averaged data indicate that when mean values of data points were calculated, marginal values that caused lower R^2 values in separate curves were pulled into the center (Figs. 1 and 2). Thus, high R^2 were obtained in average growth curves because the regression line approximated the real data points.

As shown in Table 1, *S. enterica* grew faster than *L. monocytogenes* in the temperature range tested (7–30 °C) at which MPV can be exposed from farm to fork ($p < 0.05$). Overall, *S. enterica* presented μ 1.5–3.5 times higher than *L. monocytogenes*. Koseki and Isobe (2005a, b) reported μ of approximately 0.02 and 0.04 log CFU/g/h at 10 °C, respectively, for *S. enterica* and *L. monocytogenes* growing on iceberg lettuce. In our study, the average μ of both pathogens at 10 °C was 0.045 and 0.024 log CFU/g/h, respectively. Marked differences in λ were found at low temperatures (7 and 10 °C), when *L. monocytogenes* presented larger values in comparison to *S. enterica* ($p < 0.05$). It is known that even during minimal processing, microorganisms are exposed to stressors such as cold, sanitizers, modified atmosphere and the presence of background microbiota (Capozzi et al., 2009). Even though both pathogens present several mechanisms to overcome

hurdles faced during food processing (Gandhi and Chikindas, 2007; Spector and Kenyon, 2011), *L. monocytogenes* is noticeably more prone to inhibition by indigenous microorganisms present in MPV, such as lactic acid bacteria, than is *Salmonella* (Liao and Fett, 2001; Hanning et al., 2008; Dominguez and Schaffner, 2008). Therefore, considering the similar responses of both pathogens to other stressors found during minimal processing (*i.e.*, sanitizer and modified atmosphere), it may hypothesized that the inhibitory effect of indigenous microbiota on *L. monocytogenes* supplants the ability of this pathogen to adapt to chilling temperatures (Gandhi and Chikindas, 2007; Capozzi et al., 2009). Altogether, these factors may have substantiated the faster growth of *S. enterica* compared to *L. monocytogenes*, in minimally processed lettuce under the conditions tested in the present study

Temperature is a major environmental factor affecting microbial growth kinetics in foods (McMeekin et al., 2008). Regression lines of secondary models depicted in Fig. 2 were drawn based on the mean growth kinetics parameters of *S. enterica* and *L. monocytogenes* strains while the data points added were obtained from models of each different strain of the pathogens. It can be noticed that most data points were generally within the 95% confidence interval of the averaged values. Modeling of μ was successfully performed using a linear relationship between the square root of the parameter and temperature as described by Ratkowsky et al. (1982). However, the natural logarithm transformation of lag time (λ) resulted in better fit than the square root transformation for this parameter and temperature.

Data on secondary modeling obtained herein were compared to those available in the literature (Koseki and Isobe, 2005a, b) and to tertiary models such as ComBase (Baranyi and Tamplin, 2004) and PMP. These tertiary models are the most recognized software of modeling used to predict growth parameters of several foodborne microorganisms under a variety of conditions. Literature data used for comparison were comprised of models previously described by Koseki and Isobe (2005a, b), since these are the only papers dealing with the growth kinetics of *S. enterica* and *L. monocytogenes* in MPV in the wide range of temperatures these products may be subjected from farm to fork. As can be seen in Fig. 2, secondary models derived from ComBase and PMP basically follow the same slope pattern regarding μ and λ. Overall, our λ models for *S. enterica* and *L. monocytogenes* were closer to ComBase, PMP and Koseki-derived models than our μ models (Fig. 2). Differences among our models, ComBase and the PMP-derived models can be due to the use of data obtained from culture media for building tertiary models. When growing in culture media, microorganisms are not exposed to factors such as the structure of foods and the presence of background microflora, among other issues. The differences between foods and culture media might result in the stress of bacterial cells, leading to faster μ (and greater slopes) when growth takes place in culture media. This hypothesis can be supported by the fact that μ is a characteristic of a microorganism growing in a particular environment, while λ is dependent upon the history and physiological state of the bacterial cells (Baranyi et al., 1995).

RMSE is a measure of the precision of a predictive model, and accounts for the differences between predicted and observed values. The lower the RMSE value for a model, the more precise the data were described (Ross, 1996). Therefore, the RMSE values obtained in this study are very close to those found in models available in the literature, which indicates that the models built here are generally suitable for modeling the growth of *S. enterica* and *L. monocytogenes* in MP lettuce. In addition, the current study adds to the available literature data on the growth of foodborne pathogens in MPV and can be used to build risk assessment models.

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