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Modelling the growth of *Salmonella* spp. and *Escherichia coli* O157 on lettuce

Olivier Veys[a], Susana de Oliveira Elias[b], Imca Sampers[a], Eduardo César Tondo[b]

[a]*Laboratory of Food Microbiology and Biotechnology, Department of Industrial Biological Sciences, Faculty of Bioscience Engineering, Ghent University Campus Kortrijk, Graaf Karel de Goedelaan 5, Kortrijk B-8500, Belgium*

[b]*Federal University of Rio Grande do Sul, Avenida Bento Gonçalves, 9500 – Campus do Vale – Prédio 43212 – CEP 91501-970 - Porto Alegre/RS/Brasil.*

Abstract

This study aimed to model the growth of *Salmonella* and *Escherichia coli* O157 on lettuce at different temperatures. Microorganisms were inoculated separately on lettuce and stored at 5, 10, 25, and 37°C. Growth curves were built by fitting the data to the Baranyi's DMFit model and Ratkowsky equation was used as secondary model. The models were able to assess the growth of both microorganisms and data showed that bacteria did not growth for 24 hours at 10°C, what can be a suitable temperature for lettuce distribution on food services. However, prolonged periods demonstrated growth at every temperatures examined.

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Keywords: microbial pathogens; predictive modeling; food safety; leafy greens; temperature

1. Introduction

Foodborne pathogens are a major concern in the global food market today, including *Salmonella* spp. and *E. coli* O157[1]. Currently, the production of fresh produce is associated with a healthy lifestyle, however it can be contaminated throughout the production chain and consumption. Consequently, a high percentage of the population is exposed to foodborne illnesses due to the consumption of fresh products[2], if appropriate control measures were not applied.

Lettuce is one of the most consumed leafy green cultivated around the world[2]. In Brazil, lettuces are responsible for approximately 40% of the total volume of fresh produce traded; this high consumption is attributed due to the availability, cost, and nutritional factors[3]. Besides this, independently the way lettuces are served, it is always consumed raw, being possibly to be contaminated by pathogens as *Salmonella* spp. and *E. coli* O157[2].

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The storage temperatures of lettuces before eating are highly variable, depending on the available equipment and environmental conditions. Temperature is an important extrinsic factor affecting microbial growth and, consequently, the safety of lettuces[4]. The recommended temperature for storage of ready-to-eat fresh produce in Brazilian food services is <5°C[5]. However, it is hardly reached and maintained on buffets, because of equipment used are frequently open, allowing temperature changes. Moreover, the environmental temperature in Brazil is much higher than 5°C, increasing the probability of microbial growth. Based on this, the investigation of pathogens on lettuce is important to understand the microbial behavior.

To model the influence of the temperature on the growth of *Salmonella* spp. and *E. coli* O157 primary and secondary models can be used. With these models, the behavior of pathogens can be simulated and predicted on lettuce, giving important information on the safety of these leafy greens.

2. Materials and Methods

2.1. Strains

The *Salmonella* strains used were: *Salmonella* Enteritidis SE*86*, *Salmonella* Typhimurium L12031, *Salmonella* Typhimurium 1T2, *Salmonella* Anatum, *Salmonella* Newport and *Salmonella* Saint Paul. The *E. coli* O157 strains were isolated from different sources, two were isolated from bovines in the States of Rio Grande do Sul and São Paulo, and the other two were isolated from manure and from lettuce washing water in Porto Alegre city (Brazil).

2.2. Lettuce

The lettuces were bought in a local supermarket of Porto Alegre city, Brazil. The outer leaves of the lettuces and the core were removed, as well as all visible dirtiness. The intact leaves were cut into 4 X 4 cm pieces, using a sterile surgical knife and a disinfected metallic template.

2.3. Pathogens inoculation on lettuce

Each *Salmonella* and *E. coli* O157 strain was grown in 5 mL of Brain Heart Infusion broth (BHI), at 37°C for 24h. The cultures were centrifuged, separately, at 4°C, for 10 min, at 2810*g*, the supernatants were discharged and pellets were washed with 0.1% peptone water. This procedure was repeated 3 times and then, after the third repetition, cells were re-suspended with 0.1% peptone water and all *Salmonella* and *E. coli* O157 strains were mixed in two different pools.

The final cell concentration of 10^8 CFU/mL was adjusted through optical density (OD_{630nm}) and confirmed by plating on BHI. Decimal serial dilutions using 0.1% peptone water were prepared, and a *Salmonella* pool was inoculated on lettuce in order to obtain a final cell concentration of nearly 2 log CFU/g. The *E. coli* O157 pool was inoculated on lettuces in order to reach a final concentration of nearly 4 log CFU/g.

2.4. Storage conditions and enumeration of pathogens on lettuce

Inoculated portions (10 g) of lettuces were stored at 5, 10, 25, and 37°C for different periods in stomacher bags. These temperatures were chosen because they simulate the following scenarios: recommended temperature of Brazilian regulation (<5°C), suitable fridge temperature (10°C), environmental temperature (25°C) in Brazil and the ideal growth temperature (37°C) for the bacteria[6].

Analysis was carried out at varied time intervals, depending on the storage temperature. At each time point, 10 g of sample were homogenized with 90 mL of 0.1% peptone water, followed by decimal dilution in 0.1% peptone water. Then, aliquots were plated onto Plate Count Agar (PCA) and on Xylose Lysine Deoxycholate agar for *Salmonella* and onto PCA and Sorbitol MacConkey agar, for *E. coli* O157, and incubation at 37°C for 24h. All the bacterial counts were carried out in triplicate. The experiments were repeated three times and the results were expressed as log CFU/g.

2.5. Modeling of pathogens growth on lettuce

The predictive primary model described by Baranyi and Roberts (1994)[7] was used in order to calculate the growth kinetic parameters of pathogens on lettuce. The growth curves for each temperature were built by fitting the experimental data to the Baranyi's DMFit version 2.1 Excel® add-in (www.ifr.ac.uk/safety/DMfit). The following parameters were obtained: 1) maximum growth rate (μ), 2) *lag* time (λ), and 3) maximum population density.

The predictive secondary model was built using the square root model described by Ratkowsky et al. (1982)[4] to describe μ and λ as a function of storage temperature.

2.6. Model evaluation

Measures of coefficient of determination (R^2) were used to evaluate the performance of the models built in this study. The R^2 was generally considered as an overall measure of the prediction calculated by developed model, and the closer to 1 the better the model's performance[8].

3. Results and Discussion

*Salmonella s*pp growth curves started with an initial concentration of nearly 2 log CFU/g, and reached a final concentration of 8 log CFU/g, after 10 h at 37°C. For the other temperatures, 6 log CFU/g were reached after 10, 100 and 300 h at 25, 10 and 5°C, respectively. These results are shown in Figure 1.

For the pool of *E. coli* O157, all growth curves started with an initial concentration of nearly 4 log CFU/g and reached a final concentration of 7 log CFU/g after 8 h, at 37°C, and for the other temperatures 6 log CFU/g were obtained after 6 and 150 h at 25 and 10°C, respectively. These results are shown in Figure 1.

It can be observed that the temperature had a considerable influence on the microbial behavior, because final concentrations were different. This may be explained by the enzymatic leaf browning of lettuce that has antimicrobial compounds, causing a reduction of bacteria[9] or by the lettuce microbiota that may cause competition for nutrients and space, resulting in less growth of pathogens mainly at low temperatures.

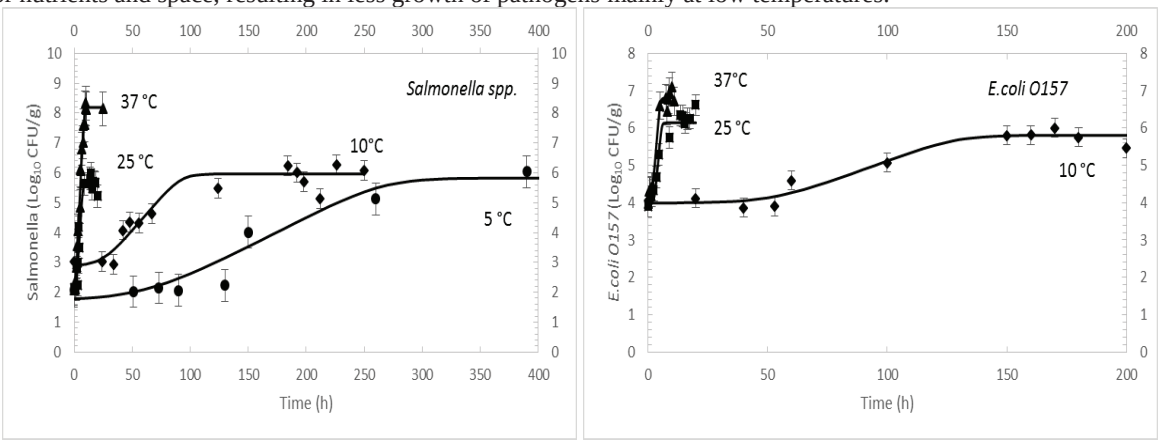

Fig. 1. The observed growth of *Salmonella* and *E. coli* O157 on lettuce stored at 5°C (symbolized by ●), 10°C (♦), 25°C (■), and 37°C (▲), fitting data to DMFit add-in version 2.1. Each symbol represents a mean of triplicate trials results.

Table 1 presents the primary growth parameters (growth rate, *lag* time and maximum population density) of pathogens on lettuce stored at different temperatures. It also shows the R^2, a measure that the closer is to 1, more the model will represent the reality. These parameters were estimated by DMFit (Table 1).

Table 1. Growth parameter of *Salmonella* and *E. coli* O157 inoculated on lettuce at different temperatures. MPD is maximum population density.

Temperature	*Salmonella*[a]				*E. coli* O157[a]			
(°C)	Growth rate (log CFU/h)	Lag time (h)	MPD (log CFU/g)	R^2	Growth rate (log CFU/h)	Lag time (h)	MPD (log CFU/g)	R^2
5	0.02	63.2	5.82	0.92	ND[b]	ND	ND	ND
10	0.05	24.6	5.96	0.93	0.02	52.1	5.80	0.93
25	0.63	1.85	5.64	0.95	0.71	2.72	6.14	0.96
37	0.82	0.85	8.2	0.99	0.79	1.80	6.80	0.97

[a] Mean value of triplicate trials.
[b] Not determined.

Based on the results of Table 1, it can be observed that there is a good fit between the experimental data and the primary model (R^2). Therefore, the data obtained in primary model (the values of μ and λ) were used to elaborate a secondary model (Table 2), which allowed the prediction of the parameters (growth rate and lag time) described on the basis of the temperature variation. The developed models were able to assess the growth of both *Salmonella* spp. and *E. coli* O157 on lettuce under various temperatures, ranging from 5 to 37°C and 10 to 37°C, respectively.

Table 2: Secondary model represented by square root equation, showing the relationship between growth rate and temperature and lag time and temperature of Salmonella and E. coli O157 inoculated on lettuce.

μ_{max} is the maximum growth rate (log CFU/g/h); λ is lag time (h); T is temperature (°C).

Bacteria	Square root equation	R^2
Salmonella	$\sqrt{\mu_{max}} = 0.027(T - 5.42)$	0.97
Salmonella	$\sqrt{\lambda} = 0.212(T + 37.15)$	0.88
E. coli O157	$\sqrt{\mu_{max}} = 0.028(T - 1.58)$	0.85
E. coli O157	$\sqrt{\lambda} = 0.224(T + 39.19)$	0.84

4. Conclusion

The developed models were suitable to assess the growth of both *Salmonella* spp. and *E. coli* O157 on lettuce stored at 5 to 37°C and 10 to 37°C, respectively. Besides this, the results of this study indicated that lettuces exposed to 10°C for until 24h did not supported the growth of pathogens investigated, suggesting that this temperature is adequate to keep lettuce exposed on buffets of restaurants and supermarkets.

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