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Growth kinetics parameters of *Salmonella* spp. in the peel and in the pulp of custard apple (*Annona squamosa*)

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Abstract

The growth kinetic parameters (maximum growth rate, μ; lag time, λ and maximum population, k) of *Salmonella* spp. on the peel and in the pulp custard apple at 10, 15, 20 and 30 °C were determinated. Samples of peel and pulp of custard apple were inoculated with a cocktail of three strains of *Salmonella* spp. (S. Typhimurium, *S*. Enteretidis and *S*. Montevideo) and further stored at 10, 15, 20 and 30ºC. *Salmonella* can survive and multiply in both, the peel and in the pulp of the custard apple. Lower temperatures retard, but do not prevent *Salmonella* growth.

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Keywords: Salmonella, custard apple, Predictive modelling, growth, tropical fruits.

1. Introduction

Occurrence of foodborne diseases has increased significantly worldwide. Data of Department Health Surveillance (SVS) of the Ministry of Health from Brazil show that between 2000 and March 2013 there were 8746 foodborne diseases outbreaks in the country, 28 cases associated with the consumption of fruits and their products [1]. However, due to failures as difficulties in tracking and reporting system, it is estimated that the combination of these products with foodborne diseases is much higher. Thus, contamination of fruits and vegetables by pathogenic microrganisms constitutes a serious public health problem.

Several pathogens such as *Escherichia coli*, *Salmonella*, *Listeria monocytogenes*, can contaminate tropical fruits. However, *Salmonella* has been the most common pathogen associated with foodborne diseases involving fruit [2, 3,

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4]. The contamination of fruits and vegetables can occur at various points in the production chain, reinforcing the need for scientific research about the microbiology of Brazilian tropical fruits.

At the exotic tropical fruits segment, particularly in Brazil, açaí, avocado, cupuaçu, jabuticaba, custard apple, breadfruit, among others stand out . Among these, the custard apple (*Annona squamosa*) has highlighted, because it has great market potential, given the diversity of functional compounds and vitamins. It is a low acid fruit with pH of approximately 5.0.

Considering the low acidity of this fruit, once in contact with the pulp, pathogenic microorganisms could find conditions to multiply and cause serious public health problems in Brazil, as well as in importing countries. This fact could cause serious problems to the national industry, as discredited as regards the safety of food produced in Brazil and as a result, huge financial losses to the country.

Thus, this study is justified by the market potential of this fruit and the lack of data on the growth kinetic parameters of *Salmonella* on custard apple, at the temperature conditions to which they are exposed from the field to the consumer. Thus, the purpose of this study was to determine the growth kinetic parameters (maximum growth rate, μ; lag time, λ and maximum population, k) of *Salmonella* spp. on the peel and in the pulp of custard apple at 10, 15, 20 and 30 ° C.

2. Material and Methods

2.1. Determination of the multiplication parameters (maximum growth rate, lag time and maximum population) of Salmonella at 10 ° C, 15 ° C, 20 ° C and 30 ° C.

The multiplication parameters (maximum growth rate, μ; lag time, λ and maximum population, κ) were determinated using a pool of three different strains of *Salmonella* spp (S. Typhimurium isolated from custard apple, *S.* Enteretidis and *S.* Montevideo). Triplicate test portions of the peel and pulp were inoculated with 10 μL of a 10^4 CFU/mL of *Salmonella* spp. The peel and pulp samples were incubated, for up to 132 h, 84 h, 48 h and 26 h, respectively at 10, 15, 20 and 30 ºC. Sampling was carried out at different intervals and was done using MLCB count agar incubated at 37 ° C / 24 h.

Growth curves for each temperature were built separately for the peel and pulp by fitting data to the Baranyi model [5] using DMFit version 2.1 Excel® add-in (www.ifr.ac.uk/safety/DMfit).

2.2. Statistical analysis

The growth kinect parameters of *Salmonella* were checked for significant statistical differences ($p \leq 0.05$), employing one-factor analysis of variance (ANOVA) followed by Tukey's test.. Statistical analyses were carried out in Assistat version 7.5 free software (Campina Grande, Brazil) [6].

3. Results and Discussion

Growth curves of *Salmonella* spp. started with an initial population of 10^2 - 10^3 CFU/g and final populations varied between 10^2 and 10^6 CFU/g, the k values showed significant differences ($p < 0.05$) between all temperatures, with the lower at 10ºC (10^2 CFU/g) and the highest at 30 ºC (10^6 CFU/g). Control samples did not show growth of *Salmonella* during storage at different times and temperatures.

Figure 1 shows the average growth kinetic parameters for *Salmonella* on the peel and in the pulp of custard apple. At 10 ºC, *Salmonella* presented highest λ , both on the peel and in the pulp in comparison to another temperatures, and the lower λ was verified for pulp at 30 ºC ($p < 0.05$).

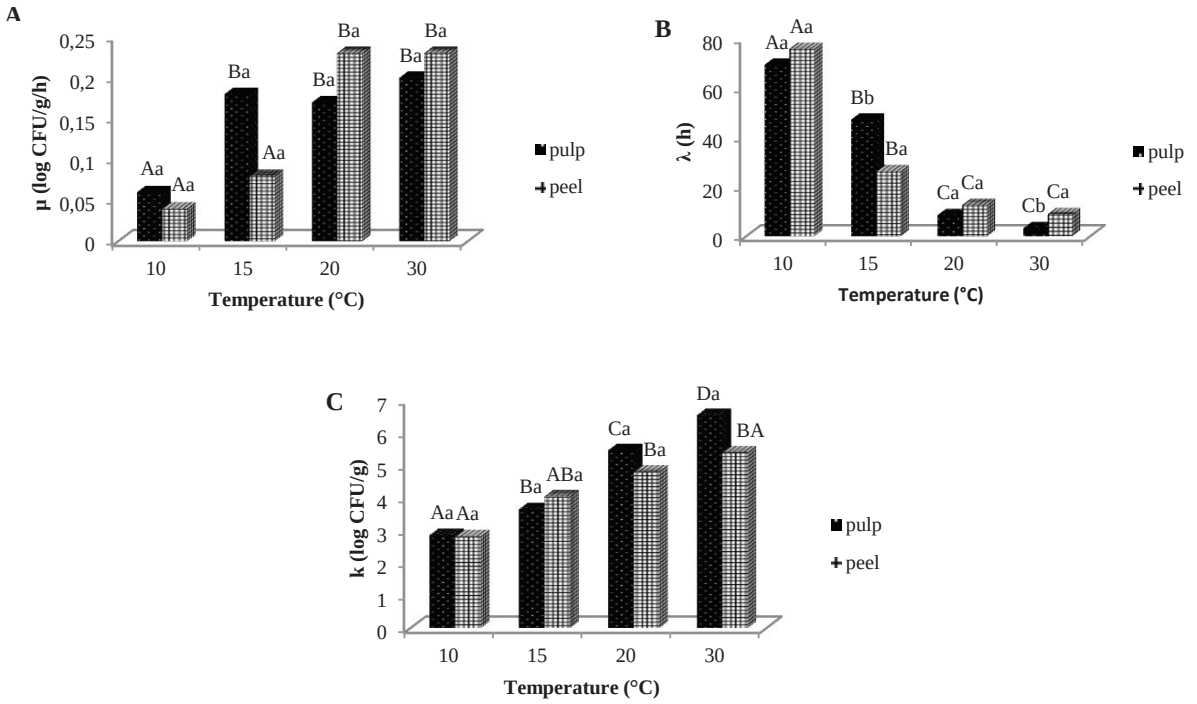

Figure 1. Growth kinetic parameters: maximum growth rate, μ log CFU/g/h (A); lag time, λ/h (B) and maximum population, k, log/CFU/g (C) of *Salmonella* spp. in the peel and in the pulp of custard apple storage at 10, 15, 20 and 30°C[ab]. [a]Different capital letters for different temperature for the pulp or the peel indicate significant differences ($p < 0.05$) according to Tukey's test. [b] Different small letters for the same temperature between pulp and peel indicate significant differences ($p < 0.05$) according to Tukey's test.

When growth parameters among pulp and peel for the same temperature were evaluated, no significant differences were observed for *Salmonella*, except for λ at 15 and 30 ºC ($p < 0.05$).

When evaluating the μ, the values differed significantly among the temperatures ($p < 0.05$) with the lower μ obtained at 10 and 15 ºC, ranging from 0.04 log/CFU/g to 0,08 log/CFU/g/h, and the highest at 20 and 30ºC, between 0.17 log/CFU/h and 0.23 log/CFU/h.

Overall, the results showed that both the pulp, as the shell of custard apple, are excellent substrates for the multiplication of *Salmonella* in all analyzed temperatures. Lower temperatures (10 ° C) retard but not inhibit the growth of *Salmonella*.

Comparing the growth of the pulp with the peel, in general, it is noted that bacterial growth was more significant in the pulp due to a higher concentration and availability of nutrients in relation to the shell. Both the shell and in the pulp, the time lag λ is inversely proportional to temperature. Being closer to the optimum temperature for pathogen multiplication, there was faster growth in both fruits at 30 ° C.

4. Conclusion

Tropical fruits such as custard apple comprise substrates that allow the growth of pathogens such as *Salmonella*, both in the pulp and also in the peel. The main implications are that even contamination of peel at pre- or post-harvest steps may lead to spread of *Salmonella* and, for example, on transference of this pathogen during washing and cutting operations, which may potentially affect public health.

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