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Modeling the fate of *Listeria monocytogenes* and *Salmonella enterica* in the pulp and on the outer rind of Canary melons (*Cucumis melo* (Indorus Group))

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ABSTRACT

The aim of this study was to develop a predictive model for the growth of *L. monocytogenes* and *Salmonella* in the pulp and the outer rind of Canary melons (*Cucumis melo* L.) as a function of temperature. The experiments of *L. monocytogenes* and *Salmonella enterica* showed that the former can grow in all temperatures tested, from 5 °C to 35 °C, both on the outer rind and in the pulp of melons while the latter did not grow when the outer rind and the pulp of Canary melons were stored at 5 °C. There was no significant difference ($p > 0.05$) regarding the growth kinetic parameters [growth rate (μ) and lag time (λ)] when the pathogens were inoculated on the outer rind and pulp following storage between 15 and 35 °C. Secondary models (Ratkowsky model) were able to describe the influence of temperature on μ and λ for both microorganisms on the outer rind and pulp of Canary melons. This study adds quantitative data to the literature on the fate of *L. monocytogenes* and *S. enterica* in Canary melons and reveal that pulp and the outer rind of this fruit comprise substrates that support the growth of both pathogens in a wide range of storage temperature.

1. Introduction

At harvest, fresh fruits normally have a microbiota mainly formed by microorganisms that are not pathogenic. However, contamination by pathogenic microorganisms can take place throughout the production chain such as at pre-harvest, post-harvest, processing, packaging, selling and during preparation for consumption (WHO, 2008; Nunes et al., 2010). Brazil, one of the largest producers of fruits in the world, generates exports of fresh fruits with revenues above US$ 609 million. Melon is the most prominent fruit in the fruit exports contributing more than 177,000 tons (IBRAF, 2015). However, occurrences of outbreaks involving pathogenic microorganisms with this fruit have raised the attention of producers, consumers and health surveillance agencies.

The outer rind of melons has a surface that bacteria can strongly adhere to (Svoboda et al., 2016; Ukuku, Mukhopadhyay, Geveke, Olanya, & Niemira, 2016). Therefore, the pulp of melon can be contaminated during cutting if the sanitation procedure is not able to inactivate pathogenic bacterial contaminants adhered to the surface. Subsequently, considering the pH (5.2–6.7) and water activity (0.97–0.99) of melons are favorable for the growth of pathogens (Fang and Huang, 2013; Danyluk, Friedrich, & Schaffner, 2014), these microorganisms may find conditions not only to survive but also to grow in these fruits.

In Brazil, data from the Health Surveillance Secretariat of the Ministry of Health of Brazil indicate that, between 2000 and 2014, there were 9719 cases of foodborne diseases. *Salmonella* spp. was the main pathogen, involved in approximately 38.02% of the cases of foodborne diseases. Fruits and fruit products have been reported in 33 cases (MS – SVS, 2014). *Salmonella* spp. stands as the most important microorganism from the public health point of view in

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Brazil (MS – SVS, 2014). The occurrence of *L. monocytogenes* in tropical fruits (Uchima et al., 2008) and vegetables (Oliveira, Abeid Ribeiro, MoratoBergamini, & Pereira De Martinis, 2010; Maistro, Miya, Sant'Ana, & Pereira, 2012; Sant'Ana, Igarashi, Landgraf, Destro, & Franco, 2012b) as well as the ability of this bacterium to grow in substrates of plant origin have been reported in Brazil (Penteado, Castro, & Rezende, 2014; Sant'Ana, Destro, Landgraf, & Franco, 2013; Walter, Kabuki, Esper, Sant'Ana, & Kuaye, 2009). In spite of this, until now no outbreak associated to foods of plant origin has been reported in the country (Vallim et al., 2015). Nonetheless, in the United States, the data from the Food and Drug Administration (FDA) indicate that, from 1996 to 2008, 82 foodborne disease outbreaks occurred associated with the consumption of produce. Among these, 13 (15.9%) were related to the consumption of melons (FDA, 2009). In 2011, an outbreak of listeriosis related to cantaloupe consumption resulted in 33 deaths and an abortion, in 28 states of the USA (CDC, 2012a). In 2012, the CDC reported an outbreak caused by the consumption of cantaloupe contaminated with *Salmonella* spp., which led three people to death and other 94 hospitalized (CDC, 2012b).

An important aspect of the *L. monocytogenes* and *Salmonella* outbreaks linked to cantaloupes is that they involved fresh whole fruits instead of minimally processed (cut) fruits (CDC, 2012a; b). Investigations were able to trace back *L. monocytogenes* and *Salmonella* strains involved in the outbreaks to the farms or packing house facilities (CDC, 2012a; b). This has highlighted the role of fruits peels (rinds) as an environment for the survival and further transmission of foodborne pathogens. As such, it is clear that not only the avoidance of microbial contamination during preparation of the fresh-cut fruit is critical for food safety, but also the contamination of the whole fruit, in all steps preceding fruit cutting. Despite this, previous studies have mainly focused on the modeling of growth of *L. monocytogenes* on fresh-cut cantaloupe (Fang and Huang, 2013) and cantaloupe, honeydew and watermelon (Danyluk et al., 2014), while two other studies have modeled the growth of *Escherichia coli* O157:H7 on cantaloupe, honeydew, and watermelon and *Salmonella* on honeydew and watermelon (Li, Friedrich, Danyluk, Harris, & Schaffner, 2013). Considering the above mentioned, data is limited on the growth of *L. monocytogenes* and *Salmonella* in the pulp of melons, and is specifically lacking regarding their growth on whole Canary melons. In addition, to the best of author's knowledge there are no data on the growth kinetics of *L. monocytogenes* and *Salmonella* on the outer rind of melons, including Canary melons. Given the association of *L. monocytogenes* and *Salmonella* with foodborne disease outbreaks linked to melons, it is important to understand their behavior not only in the pulp but also on the outer rind of these fruits. Data on the growth kinetic parameters of these pathogens in Canary melons may be important because they can be used to develop strategies to reduce the incidence of foodborne disease outbreaks associated with these fruits. Consequently, the objective of this research was to develop a mathematical model to predict the growth of *L. monocytogenes* and *Salmonella* on the outer rind and pulp of Canary melons as a function of temperature.

2. Material and methods

2.1. Determination of the growth parameters (growth rate and lag time) of L. monocytogenes and S. enterica on the outer rind and pulp of Canary melons

2.1.1. Samples

Whole Canary melons (also known as Spanish melon) (*Cucumis melo* L. (Indorus Group)) were acquired in a market and stored under refrigeration at 7 °C for a maximum of 24 h before the beginning of the experiment. Whole Canary melons were washed and sanitized for 15 min with chlorinated solution at 300 mg/L, pH = 7.0 ± 0.2. A solution of sodium thiosulphate 0.25% was used to neutralize the sanitizing agent after the treatment. Then, the whole Canary melons were cut with previously sterilized knife under aseptic conditions. Pieces of approximately 10 g ± 1 g of the outer rind and pulp were obtained. A thin portion of the outer rind was cut in order to ensure the absence of residuals of pulp.

2.1.2. Strains and inoculum preparation

A strain of *Listeria monocytogenes* serovar 1/2b (UFV_2) isolated from melon and a strain of *Salmonella enterica* subsp. *enterica* serovar Typhi (NR-074799.1) isolated from lettuce were used. With the aid of a sterile platinum loop, an aliquot of each strain, frozen at −80 °C, was transferred to Brain Heart Infusion (BHI) broth (Thermo Fisher Scientific Oxoid, Basingstoke, UK) and incubated at 35 °C ± 1°C/24 h. The cultures were transferred again to the BHI broth following incubation at 35 °C ± 1 °C for 18 h. For the standardization of the inoculum, a spectrophotometer (model Kazuaki IL-227, Kazuaki, Brazil) was used and the reads were done at the wavelength of 625 nm, according to the 0.5 McFarland standard (Rezende, Igarashi, Destro, Franco, & Landgraf, 2014; Sant'Ana, Barbosa, Destro, Landgraf, & Franco, 2012a; Sant'Ana et al., 2013).

2.1.3. Inoculation of the outer rind and pulp of Canary melons and enumeration of L. monocytogenes and S. enterica

The pH and water activity of the outer rind and pulp of melons were measured before the inoculation with the pathogens. For the analysis of pH, 10 g of the outer rind and pulp were separately weighted and diluted in 100 mL of distilled water, and the reading was carried out in pH meter (Ion PHB-500) (Instituto Adolfo Lutz, 1985, p. 27). The water activity of the samples was measured using the 4TE Dewpoint Water Activity Meter (AquaLab® Decagon, Washington, USA).

The outer rind of whole Canary melons were placed inside sterile Petri dishes with the peel portion facing up. Then, these parts of the fruit were separately inoculated with an aliquot of each pathogen aiming to reach a final count of 10^2–10^3 CFU/g. The pulp of the whole Canary melons was placed within sterile plastic bags and inoculated with each pathogen aiming to reach a final count of 10^2–10^3 CFU/g. Then, the Petri dishes and plastic bags containing the inoculated outer rind and pulp of whole Canary melons were incubated at different temperatures (5 °C, 15 °C, 20 °C, 30 °C and 35 °C). The enumeration of *L. monocytogenes* and *S. enterica* were carried out at different times, according to the incubation temperature. For this, the samples were submitted to decimal dilutions using sterile 0.1% peptone water, following homogenization in a vortex (Vortex Genie® 2 G-560, Scientific Industries, Inc., Bohemia, New York, USA) for 2 min. Then, the plating was done by the technique of microdrop using Oxford agar (Merck, Darmstadt, Germany) for *L. monocytogenes* and Hektoen Enteric agar (Thermo Fisher Scientific Oxoid, Basingstoke, UK) for *S. enterica*. Further, the plates were incubated at 35 °C ± 1°C/24 h, and typical colonies were enumerated, following expression of the results as CFU/g.

2.1.4. Predictive modeling

The experiments were repeated twice and performed in duplicate. The planned time intervals for sampling were based on data on the growth of microorganisms in broth provided by the Pathogen Modelling Program – PMP, version 7.0 (provided by the US Department of Agriculture – Agricultural Research Service USDA-ARS).

The growth curves were adjusted to the Baranyi model (Baranyi & Roberts, 1994) (Equations (1)–(3)) using DMFit, version 2.1. Excel (www.ifr.ac.uk/safety/DMFit).