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Quantifying the effectiveness of washing treatments on the microbial quality of fresh-cut romaine lettuce and cantaloupe

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ABSTRACT

The increase in foodborne illness outbreaks associated with fresh and fresh-cut produce in the USA has been attributed to ineffectiveness of current handling practices. This study describes the change in concentration of population of *Listeria innocua* to then quantify the effectiveness of washing treatments and storage temperature in the growth of *Listeria monocytogenes* in two popular fresh-cut produces, romaine lettuce and cantaloupe. *L. innocua* was used as a surrogate for *L. monocytogenes* to experimentally evaluate the effectiveness of washing treatments (water and chlorine) and develop growth curves at 5–36 °C storage. Both treatments were more effective ($p < 0.05$) in reducing *L. innocua* concentration in fresh-cut romaine lettuce than in cantaloupe. For instance, chlorinated water treatment reduced *L. innocua* population by 0.98 log on fresh-cut romaine lettuce compared to just 0.57 log on cantaloupe rind. The experimental data on *L. innocua* were used to test the Baranyi-Roberts model in both produce and results demonstrate that it can be a useful tool to estimate the growth of *L. monocytogenes* in selected fresh-cut produce during distribution, storage or at the market, or at home using quantitative risk assessment methods.

1. Introduction

Fresh produce can be contaminated with pathogens in field or post-harvest applications. Although the prevalence of *L. monocytogenes* in fresh produce is relatively lower than other common pathogens, a higher mortality rate makes this pathogen a serious health problem (Behravesh et al., 2011; Beuchat, 1996; Bowen, Fry, Richards, & Beauchat, 2006; Cartwright et al., 2013; Lorber, 2007; Ryser & Marth, 2007). Cantaloupe (*Cucumis melo* L.) and bagged salads containing romaine lettuce (*Lactuca sativa*, var *longifolia)* have recently been linked to cases of listeriosis (CDC, 2016; Macarisin et al., 2017; McCollum et al., 2013; Walsh, Bennett, Mahovic, & Gould, 2014). The lack of heat treatment in the process, and the ability of *L. monocytogenes* to grow at low temperatures, increase the need to understand the growth behavior and treatment response of this pathogen.

Growth patterns are often described with mathematical models which are useful for manufacturers to develop process controls to reduce the risk of pathogen contamination in their foods. These predictive models also provide an estimate of the product's shelf-life based on microbial safety. *L. monocytogenes* cannot be safely studied in every environment because of the risk of exposure of vulnerable individuals. Consequently, the use of a surrogate organism is a convenient way to understand the behavior of this pathogen in food processing environments (Danyluk, Friedrich, & Schaffner, 2014; Milillo et al., 2011). *L. innocua* is used as a surrogate for *L. monocytogenes* due to their close genetic relationship (Guo, Jin, Scullen, & Sommers, 2013).

Several growth models have been developed to provide reliable estimates of two parameters which are characteristic of bacterial growth, the lag-time (λ) and the maximum growth rate (μ_{max}). Three models that have been used frequently in the field of predictive microbiology are the Gompertz, the Logistics, and the Baranyi-Roberts models (Buchanan, Whiting, & Damert, 1997; Perez-Rodriguez and Valero, 2013). Baty and Delignette-Muller (2004) evaluated these three models and concluded that the Baranyi-Roberts was the most consistent model since it provided the best fit for a majority of datasets and gave reasonably precise λ estimates.

Therefore, the objectives of this study were to (1) predict the growth of *L. innocua* on fresh-cut romaine lettuce and cantaloupe as a function of storage temperature by using Baranyi-Roberts model,

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(2) compare and validate the goodness of fit of the primary model with statistical validation tools, (3) determine the effect of washing treatments on the reduction of *L. innocua* on both produces and, (4) develop and validate dynamic models for prediction of growth of *L. innocua* under different storage temperatures. These results can be used as input in quantitative risk assessment models.

2. Materials and methods

2.1. Food material

Fresh-cut romaine lettuce (*Lactuca sativa*, var *longifolia*) was purchased from a local store. All products had the same shelf-life date to ensure uniformity and were stored in the original package at 5 °C for no longer than 24 h prior to experiments. All products were examined and the hearts showing signs of wilt and decay discarded. 5 g of produce was weighted and dispensed into sterile stomacher bags (18 oz. Whirl Pak® bag) before inoculation as described by Puerta-Gomez, Moreira, Kim, and Castell-Perez (2013).

Whole cantaloupes (*Cucumis melo* L.), free of visual defects, were randomly purchased from a local store in the summer season and stored at 5 °C for less than 24 h. Before the experiments, each cantaloupe was washed with tap water for 3 min and then the rind and the core were removed with a sterile knife. The fruits were subdivided into 5 g cubic shaped portions and dispensed into a sterile stomacher bag (18 oz. Whirl Pak® bag) before inoculation.

2.2. Experimental

2.2.1. Initial natural microbiota enumeration

Aerobic mesophilic bacteria were enumerated by spread plating on Tryptic Soy Agar (TSA) incubated at 36 °C for no more than 48 h. Yeasts and molds were quantified by spread plating on Sabouraud Dextrose Agar (pH 5.6, adjusted with 1 g/L citric acid) after 5 d of incubation at 20 °C (VWR International, Model 1510E, IL, USA). Plate counts of total aerobic organisms, yeasts, and molds will be evaluated only at the beginning of every experiment.

2.2.2. Inoculation and preparation of fresh-cut cantaloupe and fresh-cut lettuce

A previous study from our laboratory (Omac, Moreira, Castillo, & Castell-Perez, 2015) validated the use of *L. innocua* as a surrogate of *L. monocytogenes* for growth modeling in baby spinach leaves. Hence, we used *L. innocua* to quantify growth behavior of the pathogen in fresh-cut romaine lettuce and cantaloupe.

For each produce, 5 g portions were distributed into sterile stomacher bags (18 oz. Whirl Pak® bag), and an initial inoculum (0.5 mL) was applied. The initial bacteria load was 10^2 CFU/ml *L. innocua* to mimic natural contamination (Omac et al., 2015). To dispense the inoculum uniformly, stomacher bags were shaken gently about 30 times. Different sampling times were determined for each temperature (5, 10, 25, 30, and 36 °C) and four bags of inoculated samples were prepared for each sampling time, placed in an incubator, and maintained at constant temperature for 16 d, 12 d, 60 h, 48 h and 36 h at 5, 10, 20, 30, and 36 °C, respectively. The experiments were performed in triplicate (n = 12).

2.2.3. Bacterial cultures

Rifampicin resistant (80 μg/mL) culture of *L. innocua* (NRCC B33076) was obtained from a stock laboratory (Biological and Agricultural Engineering, Texas A&M University) stored at −80 °C. A loop was used to take a single inoculum from the frozen culture. Optimal temperature for incubation of *L. innocua* is around 37 °C (Ryser & Marth, 2007). Inoculum was put onto Tryptose Phosphate Broth (TPB; Difco, Detroit, MI), on which the inoculum was incubated for 24 h at 36 °C. Next, the inoculum was taken with a loop and streaked on Oxford Listeria-selective agar supplemented with 80 μg/mL of rifampicin (OLR) in order to obtain single colony isolates. Inoculum was incubated at 36 °C for 24 h, and this process was repeated through two successive transfers on (OLR). Obtained colonies were rifampicin resistant. These colonies were kept on a TSA slant at 5 °C, and were used in 90 d.

2.2.4. Inoculum preparation

The inoculum in TSA slant was taken with a loop, and transferred to TPB test tubes. The inocula in TPB tubes were incubated at 36 °C for 18 h. After 18 h, incubated inocula were centrifuged (3000 × g for 15 min) and washed with Difco buffered peptone water for three times consecutively. Afterwards, each pellet was suspended in 1 g/L peptone water (PW). To determine the initial concentration, the OD600 of the cell suspensions was adjusted to 0.5 of absorbance for bacterial preparation. Serial dilutions of the suspension were made in test tubes of 9 mL PW, in order to verify initial concentration will be 10^7 CFU/mL. Subsequently, the suspension was plated on OLR, and incubated at 36 °C until countable visible black colonies were obtained. To acquire 10^2 CFU/mL of *L. innocua* and strains, a series of dilutions of initial population in PW were prepared.

2.2.5. Washing and sanitizing treatments

To simulate standard industrial practices, romaine lettuce was washed after cutting and cantaloupe was washed as whole. Four bags of lettuce samples (5 g per 18 oz Whirl Pak® bag) with different initial inoculum loads (10^3, 10^4, and 10^5 CFU/mL) of *L. innocua* were washed with tap water for 10 min at room temperature. During the treatment, the washing solution was sometimes stirred to increase the water contact. Then, four different bags of samples (5 g per sample in 18 oz Whirl Pak® bag) with same initial loads as for water washing were treated with 200 mg/L of chlorinated water at pH 7.0 (reduced with 0.1 g/L HCl) for 10 min at room temperature. Similarly, the solution was stirred occasionally during the treatment. After the treatment, each 5 g sample was placed in an 18-oz. stomacher bag and kept at 5 °C for 2 h (Omac et al., 2015). Then, the number of microorganisms remaining on the surface of the products was determined using microbial enumeration methods.

Washing of cantaloupe was carried out as described by Vadlamudi, Taylor, Blankenburg, and Castillo (2012). 500 mL bacterial solutions were prepared as described above and transferred into a bowl containing 4500 mL of 1 g/L peptone water solutions to produce 5 L of solution. Whole cantaloupes were submerged into the solution for 3 min and gently agitated with gloves. After every sample, gloves were changed to eliminate the risk of cross contamination. The fruit samples were allowed to dry at room temperature in a biosafety cabinet (Labconco Purifier Logic Class II Type 2, Kansas City, MO) for 2 h prior to the washing treatments. Sanitizers were prepared as described before. All samples (including those subject to chlorine washing) were washed for 3 min under tap water. After that, chlorine washing samples were submerged into 5 L of a 200 mg/L chlorine solution, and rotated for 5 min. Next, five samples were collected with a sterile cork borer (1 cm^2 area), and a sterile scalpel and placed inside an 18 oz Whirl Pak® bag (Nasco, Fort Atkinson, WI) containing 99 ml of 1 g/L PW. Series of dilutions were prepared to enumerate the *L. innocua* cells. The colonies were divided by 5 to determine the count in CFU/cm^2.

2.2.6. Microbial enumeration

Each 5 g sample of fresh produce inoculated with *L. innocua* was hand pummeled with 45 mL of Difco buffered peptone water (BPW;