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**Research Paper**

# Predicting the Growth of *Listeria monocytogenes* and *Salmonella* Typhimurium in Diced Celery, Onions, and Tomatoes during Simulated Commercial Transport, Retail Storage, and Display

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## ABSTRACT

Temperature is arguably the most important factor affecting microbial proliferation in fresh-cut produce. In this study, growth of *Listeria monocytogenes* in diced onions and celery and *Salmonella* Typhimurium in diced tomatoes was determined in modified atmosphere packages and snap-fit containers using three fluctuating temperature scenarios for transport, retail storage, and display. As expected, *L. monocytogenes* growth in diced onions and celery varied depending on the extent of temperature abuse, with exposure to high and intermediate temperature-abuse scenarios generally being growth supportive. A Baranyi primary model with a square-root secondary model for maximum growth rate, and a linear model for maximum population density, were used to estimate *Listeria* growth under fluctuating temperature. Accuracy and acceptability of the model prediction were evaluated in terms of root mean square error (RMSE) and acceptable prediction zone (APZ), respectively. Overall, growth predictions for *L. monocytogenes* were more accurate for celery (RMSE, 0.28 to 0.47) than onions (RMSE, 0.42 to 1.53) under the fluctuating temperature scenarios tested. However, both predictions yielded APZ values that ranged from 82 to 100% for celery and 36 to 78% for onions. In contrast, *Salmonella* Typhimurium populations increased more than 1 log CFU/g in diced tomatoes under the three fluctuating temperature scenarios studied. Overall, these diced products packaged under a high-oxygen atmosphere showed decreased pathogen growth compared with product stored in a passive modified atmosphere. Findings from this study will be particularly useful in assessing the risk associated with consumption of diced celery, tomatoes, and onions and in designing effective packaging strategies to minimize pathogen growth in fresh-cut produce.

Key words: Celery; *Listeria monocytogenes*; Onions; Predictive modeling; *Salmonella* Typhimurium; Tomatoes

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Fresh-cut produce has become widely popular, with estimated sales in 2013 of approximately $27 billion accounting for 16% of total retail produce sales *(13)*. Unfortunately, an increase in recalls and foodborne illness outbreaks has coincided with the increased consumption of fresh fruits and vegetables *(38)*. Improvements in pathogen detection methods and outbreak surveillance systems are partly responsible for the reported rise in foodborne outbreaks, with the proliferation of pathogens in fresh-cut produce recognized as a considerable food safety burden.

Diced onions, celery, and tomatoes have been implicated in outbreaks of illness and recalls *(6)*. In October 2010, 10 cases, including five deaths, were reported in Texas from consumption of chopped celery contaminated with *Listeria monocytogenes (11)*. In 2012, random samples from one brand of retail diced onions and celery yielded *L. monocytogenes,* which resulted in the nationwide recall of more than 750,000 lb (340,194 kg) of diced product, along with scores of other products across the United States and Canada that included diced onions or celery as ingredients *(12)*. The following year, more than 900,000 lb (408,233 kg) of diced tomatoes were recalled from one manufacturer because of the presence of *L monocytogenes (34)*. Diced Roma tomatoes containing *Salmonella* were recalled by a different firm in 2014 *(35)*. The food safety risks associated with fresh-cut produce are particularly high, because operations such as cutting, peeling, slicing, and other tissue-damaging steps serve as viable modes of contamination *(30, 32, 36, 37)*. Therefore, effective cold-chain management during postprocess handling is critical to minimize microbial growth.

Growing evidence suggests that fresh-cut produce can experience abusive temperatures during commercial transport, retail storage, and display. Koseki and Isobe *(22)* found that the inner temperature of lettuce ranged from 3 to 15°C during transport from the processor to retail in Japan, permitting *Escherichia coli* O157:H7 and *L. monocytogenes*

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populations to increase approximately 1 and 2 log CFU/g in inoculated samples, respectively, while *Salmonella* showed no significant growth *(22)*. In other studies, temperatures during commercial transport of fresh-cut produce were generally below 10°C *(7, 24, 26)*. Instances of temperature mismanagement have been documented during retail storage and display of fresh-cut produce. In a study of fresh produce conducted at multiple retail locations across the United States, Brown et al. *(8)* reported a temperature range of 0.6 to 15.4°C during retail storage and –1.1 to 9.7°C during retail display. Simulation of some of these same temperature histories in the laboratory by Zeng et al. *(39)* led to *E. coli* O157:H7 and *L. monocytogenes* population increases of up to 3.1 and 3.0 log CFU/g, respectively, in inoculated romaine mix salad. While providing valuable information surrounding potential temperature abuse, these growth studies were conducted separately for transportation, retail storage, and display of fresh-cut products, and the potential impact of different packaging technologies was investigated.

Mathematical modeling provides a means to predict pathogen growth in various fresh-cut products under dynamic temperature conditions. When used in combination, the Baranyi primary microbial growth model, Ratkowsky secondary model, and maximum population density (MPD) equation can reliably predict *L. monocytogenes, E. coli* O157:H7, and *Salmonella* growth on lettuce stored using real-time–temperature scenarios obtained during commercial distribution *(22)*. Mishra et al. *(25)* developed growth and survival models for *Salmonella* and *L. monocytogenes* in leafy greens under nonisothermal temperatures from published data. Similarly, a combined growth-death model developed by McKellar and Delaquis *(23)* from published data on growth of *E. coli* O157:H7 in leafy green vegetables successfully predicted pathogen behavior under both isothermal and nonisothermal conditions. The McKellar and Delaquis model predictions for growth of *L. monocytogenes* and *E. coli* O157:H7 in lettuce reflected the laboratory findings by Zeng et al. *(39),* in that populations of *E. coli* and *L. monocytogenes* increased a maximum of 3.1 and 3.0 log CFU/g, respectively, in fresh-cut romaine mix during 3 days of simulated retail storage.

Most prior studies modeling pathogen growth in fresh produce under real fluctuating temperature conditions have focused on leafy vegetables. Consequently, this study aimed to (i) determine the growth of *L. monocytogenes* in packaged diced onions and celery and *Salmonella* Typhimurium in packaged diced tomatoes under simulated time-temperature conditions encountered during commercial transport, retail storage, and display; (ii) assess the impact of modified atmosphere packaging on growth of the stated pathogens under the same conditions; and (iii) predict the growth of *L. monocytogenes* in packaged diced onion and celery under dynamic temperature conditions using the Baranyi primary microbial growth model in combination with secondary microbial growth models and the calculated parameters.

## MATERIALS AND METHODS

**Construction of fluctuating time-temperature scenarios during transport, retail storage, and display.** Growth of *L. monocytogenes* in diced onions and celery and *Salmonella* in diced tomatoes was assessed under three fluctuating temperature scenarios based on time-temperature histories described in previous studies *(8, 39)*. Transportation time-temperature data were obtained from Brown et al. *(7),* who measured the temperature inside commercial refrigerated trailers during cross-country transport of bagged salad via five routes. Temperatures were collected at 5-min intervals using TempTale sensors (Sensitec Inc., Beverly, MA). Three time-temperature profiles for transport that supported *L. monocytogenes* increases of 0.5 log (scenario A), 0.3 log (scenario B), and 0.2 log (scenario C) in fresh-cut romaine mix after 48 h were chosen from this study.

Retail storage and display time-temperature data were obtained from Brown et al. *(7),* who monitored 17 retail stores in California (1), Nevada (3), Kansas (3), Ohio (3), Georgia (3), Pennsylvania (3), and New Jersey (1) for up to a year. Temperatures were recorded at four strategic locations in cold storage backrooms at 15-min intervals using TempTale sensors (Sensitec Inc.). PakSense Ultra Compact Labels (PakSense, Boise, ID) were placed in the open-front, customer-accessible displays in these same stores to record temperatures at 5-min intervals during summer and winter. In all, 2,727,340 backroom storage and 2,737,368 display time-temperature measurements were recorded. After calculating the average temperature recorded by each sensor during retail storage and display, three time-temperature scenarios corresponding to the 100th, 95th, and 90th upper percentiles were identified. Finally, three 10-day-long time-temperature profiles were created—one each for the 100th (scenario A), 95th (scenario B), and 90th (scenario C) upper percentiles—by combining the time-temperature profiles for two consecutive days of transport as described, with the temperature-time profiles for four consecutive days each of retail storage and display.

**Bacterial strains and culture preparation.** Three avirulent *L. monocytogenes* strains—M3, J22F, and J29H (provided by Dr. Sophia Kathariou, North Carolina State University, Raleigh)—and one avirulent *Salmonella* Typhimurium LT2 strain (provided by Dr. Michelle Danyluk, University of Florida, Gainesville) were used for product inoculation. Based on previous work, these avirulent strains were similar to virulent strains in terms of both attachment and growth *(32)*. Stock cultures were maintained at –80°C in Trypticase soy broth containing 0.6% (w/v) yeast extract (TSB-YE; Difco, BD, Sparks, MD) and 10% glycerol (Mallinckrodt Baker, Inc., Phillipsburg, NJ). To prepare the working cultures, each strain was streaked onto Trypticase soy agar containing 0.6% yeast extract (TSA-YE; Difco, BD) and incubated at 37°C for 24 h. A single colony of each strain was then inoculated into 9 mL of TSB-YE and incubated at 37°C for 24 h. From these cultures, 1 mL was transferred into 50 or 100 mL of TSB-YE and incubated at 37°C for 24 h. Thereafter, the three *L. monocytogenes* cultures were combined in equal volumes to obtain a three-strain cocktail, from which 30- and 75-mL aliquots were withdrawn and diluted in 30 L of tap water (7°C) to inoculate diced onions and celery, respectively. The *Salmonella* Typhimurium inoculum was prepared by diluting 50 mL of the culture in 30 L of tap water (7°C).

**Dicing and inoculation of onions, celery, and tomatoes.** Celery, jumbo yellow onions, and Roma tomatoes were purchased from a local retailer (Stan Setas, Lansing, MI) and held in a walk-

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in cold room at 4°C for no more than 24 h before use. Celery was visually inspected for defects, washed in cold water (7°C) to remove dirt, and then diced in 4.5-kg batches using a manual dicer (Nemco Slicer Model 55500-2, 9.5-mm blade grid, Nemco Food Equipment, Inc., Hicksville, OH). Jumbo yellow onions were examined for defects, peeled, and then cut to remove the tops and root ends. Onions and Roma tomatoes (5 kg) were diced in batches of 4.5 and 5.0 kg, respectively, using a commercial dicer (Urschel, model HA, Valparaiso, IN). Both diced products were then placed in mesh bags. Onions and celery were immersed in the *Listeria* inoculum for 2 and 10 min, respectively, whereas tomatoes were immersed in the *Salmonella* inoculum for 2 min. Immediately after inoculation, the three diced products were drained for 8 min, immersed in 30 L of tap water at 7°C containing 80 ppm of free chlorine (XY-12, Ecolab, St. Paul, MN; adjusted to a pH of about 6.0 with citric acid), and then dried using a 22.7-kg capacity centrifugal Spin Dryer (model SD50-LT, Heinzen Manufacturing Inc., Gilroy, CA), with three internally timed spin cycles totaling 60 s. Other batches of diced but uninoculated product were immersed in water for 2 min and then centrifugally dried for subsequent monitoring of the $O_2/CO_2$ ratio inside packages.

**pH measurement.** Approximately 50 g of knife-chopped (uninoculated) onions, celery, or tomatoes were transferred into a sterile Whirl-Pak filter bag (1.7 L, Nasco, Fort Atkinson, WI) containing 25 mL of deionized water and were homogenized for 2 min using a Stomacher 400 circulator (Seward, London, UK) at 300 rpm. A calibration check microprocessor pH meter (HI 221, Hanna Instruments, Woonsocket, RI) was then used to obtain the average pH of each homogenate from two separate readings.

**Packaging of inoculated diced produce.** Two types of polylactic acid packages were used: snap-fit containers measuring 15 by 12.5 by 5 cm and approximately 350 μm in thickness (GF 12R, GreenGood USA, La Mirada, CA) and bags measuring 11 by 12.5 cm and approximately 430 μm in thickness that were formed by impulse sealing (AIE-200, American International Electric, City Of Industry, CA) polylactic acid film (EVLON EV-HS1, BiAx International Inc., Wingham, Ontario, Canada). This film previously yielded $CO_2$ and $O_2$ permeability coefficients at 23°C and 0% RH of 30.34 ± 9.07 and 5.67 ± 1.17 × $10^{-18}$ $kg \cdot m \cdot m^{-2} \cdot s^{-1} \cdot Pa^{-1}$, respectively *(17)*. Both package types were filled with 100 g of diced onions, celery, or tomatoes. One set of 15 polylactic acid bags for each of the three products was impulse sealed under ambient air to obtain passive modified atmosphere packages (PMAPs). Another set of 15 polylactic acid bags for each of the three products was similarly sealed inside a glove box chamber (Labconco 50004 Fiberglass Glove Box, Kansas City, MO) flushed with 99% $O_2$ plus 1% $N_2$ (Airgas, Lansing, MI) for 30 min to obtain active modified atmosphere packages (AMAPs) containing about 94% $O_2$. In addition, a set of 10 snap-fit containers for each of the three products was nonhermetically sealed under ambient air (SN). Uninoculated produce samples were similarly packaged to monitor in-package gas composition.

**Incubation under simulated temperature conditions.** All packaged samples were stored for 10 days under conditions simulating temperature fluctuations during commercial transport, retail storage, and display. The time-temperature profiles for scenarios A, B, and C were separately programed into a Thermo Forma Environmental Chamber (model 3851, Thermo Fischer Scientific Inc., Waltham, MA) and monitored at 5-min intervals using a HOBO data logger (UX100-001, Onset Computer Corporation, Bourne, MA). The programing capacity of the environmental chamber used was limited to 20 time-temperature entries per cycle. Therefore, the time-temperature profiles to which the products were subjected more than 10 days were entered in multiple sets of 20, with the terminal temperature maintained until a new set of 20 time-temperature profiles could be entered. Overall, each scenario required the daily entry of four to eight sets of 20 time-temperature profiles during the 10-day storage period. Differences between the programmed transport-storage-display time–temperature data ($Y_{act}$) and time-temperature data from the incubator ($Y_{lab}$) were determined based on root mean square error (RMSE) and bias *(39)*.

**Analysis of gas composition inside packages.** For the three time-temperature scenarios, uninoculated AMAP and PMAP samples were monitored for changes in package headspace composition during storage. Oxygen and $CO_2$ concentrations were determined using a gas chromatograph equipped with a paramagnetic $O_2$ detector (series 1100, Servomex Co., Sussex, UK) and an infrared $CO_2$ detector (ADC 255-MK3, Analytical Development Co., Hoddesdon, UK) connected in series. Using a syringe (BD, Franklin Lakes, NJ), 100-μL headspace samples were withdrawn through an adhesive silicone septum, which was affixed to the package before sampling. Different packages were used for each sampling day. Gas chromatography could not be used to test the headspace of the inoculated samples because of pathogen concerns. Therefore, a leak-detection test was performed to ensure that the packages analyzed for microbial growth were undamaged and properly sealed. The method involved immersing the bags in water, applying pressure to the bags with both hands, and watching for gas bubble emission from improperly sealed or damaged packages. This method was evaluated using gas chromatography, as follows. The headspace of seven properly and seven improperly sealed $O_2$-flushed packages containing uninoculated samples was monitored using gas chromatography and the leak-detection test for 3 days. All improperly sealed packages or packages with pinholes lost their high-oxygen atmosphere after 24 h and consistently showed bubbles under water, whereas uncompromised packages did not generate bubbles under water. Overall, 1 of 15 PMAPs and 4 of 15 AMAPs failed the integrity test and were not used for subsequent analyses.

**Microbial analyses.** Bacterial populations in the inoculum suspension were determined by spread plating appropriate serial dilutions in phosphate-buffered saline (PBS) on modified Oxford agar (Neogen, Lansing, MI) for *L. monocytogenes* or bismuth sulfite (Neogen) for *Salmonella* Typhimurium, followed by 48 h of incubation at 37°C. One package for each combination of diced product (onion, celery, or tomato) and packaging type (AMAP, PMAP, or SN) was collected every 24 h, checked for proper sealing, and analyzed for numbers of *L. monocytogenes* or *Salmonella* Typhimurium, as well as mesophilic aerobic bacteria (MAB) and yeast and mold (YM). From each package (AMAP, PMAP, or SN), 25 g of diced product was aseptically transferred to a sterile Whirl-Pak filter bag (1.7 L; Nasco) and homogenized for 1 min in 75 mL of sterile PBS using a Stomacher 400 circulator (Seward) at 300 rpm. After appropriate serial dilutions, a 100-μL aliquot was spread plated on modified Oxford agar (Neogen) to enumerate *L. monocytogenes* in the diced onions and celery or on bismuth sulfite (Neogen) to enumerate *Salmonella* Typhimurium in diced tomato. Plates were incubated for 48 h at 37°C. Similarly, TSA-YE and potato dextrose agar (Neogen) were used to determine populations of MAB (48 h at 37°C) and YM (7 days at 23°C), respectively.

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**Statistical analysis.** All assay results from triplicate experiments were expressed as the mean ± standard deviation. Growth data were entered into an Excel 2010 spreadsheet (Microsoft, Redmond, WA), log transformed, and plotted against time to generate growth curves. Growth of *L. monocytogenes* and *Salmonella* in the fresh-cut products was analyzed using the paired-sample Student's $t$ test at $\alpha = 0.05$ (SPSS version 22, IBM Corporation Software Group, Somers, NY). Microbial growth variations for identically packaged products stored under different temperature conditions and packaging systems were analyzed using one-way analysis of variance, with Tukey's test used to compare multiple means ($\alpha < 0.05$).

**Growth model and parameter estimation.** *L. monocytogenes* growth in diced onion and celery was estimated using a Baranyi primary microbial growth model, with a square-root secondary model for maximum growth rate and a linear model for MPD *(2, 4)*, an approach applied in similar applications *(22, 39)* and justified by prior demonstration of no significant difference in accuracy among alternative primary models (Baranyi, Gompertz, or three-phase linear model) *(9)*. Thus, this study used the Baranyi primary model shown in the following coupled set of differential equations (equation 1):

$$\frac{dq}{dt} = \mu_{\max} \cdot q$$

$$\frac{dy}{dt} = \mu_{\max} \frac{q}{1+q}\left(1 - \frac{y}{y_{\max}}\right)y \tag{1}$$

where $y(t)$ is the natural log of the bacterial concentration, $q(t)$ quantifies the physiological state of the population, $\mu_{\max}$ is the maximum specific growth rate, and $y_{\max}$ is equal to MPD. The initial conditions for these equations are fixed as $q(0) = q_0$ and $y(0) = y_0$. Secondary models (maximum growth rate and MPD) are shown in equations 2 and 3:

$$\sqrt{\mu_{\max}} = b(T - T_{\min}) \tag{2}$$

$$y_{\max} = \beta_0 + \beta_1 T \tag{3}$$

where $T$ is the temperature, $T_{\min}$ is the conceptual lowest temperature for microbial growth, and $b$, $\beta_0$, and $\beta_1$ are constants. Baranyi and Roberts solved the preceding microbial growth primary model under a constant environment (i.e., isothermal growth), and provided the explicit solution for equations 4 and 5 *(4, 5)*:

$$y = y_{\max} - \frac{1}{m}\ln\left(1 + \frac{e^{m(y_{\max}-y_0)} - 1}{e^{m\mu_{\max}A(t)}}\right) \tag{4}$$

or, after rearrangement,

$$y = y_0 + \mu_{\max}A(t) - \frac{1}{m}\ln\left(1 + \frac{e^{m\mu_{\max}A(t)} - 1}{e^{m(y_{\max}-y_0)}}\right) \tag{5}$$

where

$$A(t) = t + \frac{1}{\nu}\ln\left(e^{-\nu t} + e^{-h_0} - e^{-\nu t - h_0}\right)$$

In equation 5, $h_0$ is a physiological state parameter, $h_0 = -\ln(q_0/(1+q_0)) = -\ln \alpha_0$, and $\nu$ and $m$ are curvature parameters *(22)*. Because the curvature parameter $\nu$ determines the curvature from the lag to the stationary phase, the constant can be assumed to be the maximum growth rate ($\nu \approx \mu_{\max}$). According to the suggestion of Baranyi et al. *(5)*, $m = 1$ (logistic curve) was applied in this study because the parameter only represents the transition from exponential to stationary phase, which is less significant in the microbial food safety *(4)*.

Using equations 4 and 5, the model parameters ($b$, $T_{\min}$, $\beta_0$, $\beta_1$, $h_0$, and $\nu$) were globally determined using an optimization algorithm (generalized reduced gradient nonlinear method; Solver in Excel, Microsoft) and the experimental growth data for *L. monocytogenes* in diced onions and celery when packaged in Whirl-Pak bags (11.0 by 22.5 cm; Nasco) and incubated at constant temperatures of 12, 16, and 23°C. The goal of the optimization algorithm was to minimize the RMSE of the entire calibration data set, as shown in equation 6:

$$\text{RMSE} = \sqrt{\sum_{i=1}^{n}\left(Y_{\text{obs},i} - Y_{\text{pred},i}\right)^2/(n-p)} \tag{6}$$

where $Y_{\text{obs}}$ is the measured log reduction, $Y_{\text{pred}}$ is the predicted log reduction, $n$ is the total number of observations, and $p$ is the number of model parameters.

To validate the model, *L. monocytogenes* growth in SN-packaged products exposed to dynamic time-temperature profiles reflecting actual transportation, retail storage, and display was predicted using the primary model (equation 5), secondary models (equations 2 and 3), and parameters described earlier, and the results were compared with the actual growth data. Growth data for *L. monocytogenes* in SN-packaged diced onions and celery were used to validate the model, because the atmospheres inside the SN packages and Whirl-Pak bags were not modified.

Accuracy of the model prediction was calculated in terms of RMSE, with model acceptability evaluated using the acceptable prediction zone (APZ) method *(28, 29)*. Prediction errors (PEs) were calculated using the equation $\text{PE} = O_e - P_v$, where $O_e$ is the observed experimental data and $P_v$ is the predicted value in log scale. Negative PE values are considered fail-safe predictions, whereas positive PE values are considered fail-dangerous predictions. Therefore, $-1.0$ log < acceptable PE < 0.5 log was used as an appropriate APZ because of the wider margin in the fail-safe direction, which is more reasonable for food safety modeling *(31)*. Based on APZ, an overall measure of model performance was calculated using the following formula: $\%\text{PE} = (\text{PE}_{\text{in}}/\text{PE}_{\text{total}})/100$, where $\text{PE}_{\text{in}}$ is the number of PE in the APZ and $\text{PE}_{\text{total}}$ is the total number of PE, with %PE > 70% considered an acceptable or valid prediction *(28)*.

## RESULTS

Throughout this study, growth data are expressed as average values ± standard deviations from triplicate trials unless otherwise stated. The *L. monocytogenes* suspensions used to inoculate diced onions and celery contained 6.2 ± 0.7 and 8.1 ± 1.1 log CFU/mL, respectively, whereas the *Salmonella* suspension for diced tomatoes contained 6.4 ± 0.3 log CFU/mL. The more concentrated cell suspension was needed for diced celery because of decreased adhesion *(21)*. After dip inoculation, diced onions and celery yielded *L. monocytogenes* populations of 4.2 ± 1.2 and 4.8 ± 0.9 log CFU/g, respectively, before sanitization. These populations decreased to 3.5 ± 0.3 and 3.3 ± 0.4 log CFU/g in diced onions and celery, respectively, after the sanitizer treatment. The *Salmonella* population for diced tomatoes, which was 4.4 ± 0.7 log CFU/g after inoculation, decreased to 3.7 ± 0.3 log CFU/g after treating with sanitizer. The pH for diced onions and celery was 6.3 ± 0.1 and 5.6 ± 0.2, respectively. The pH for tomatoes ranged between 3.9 and

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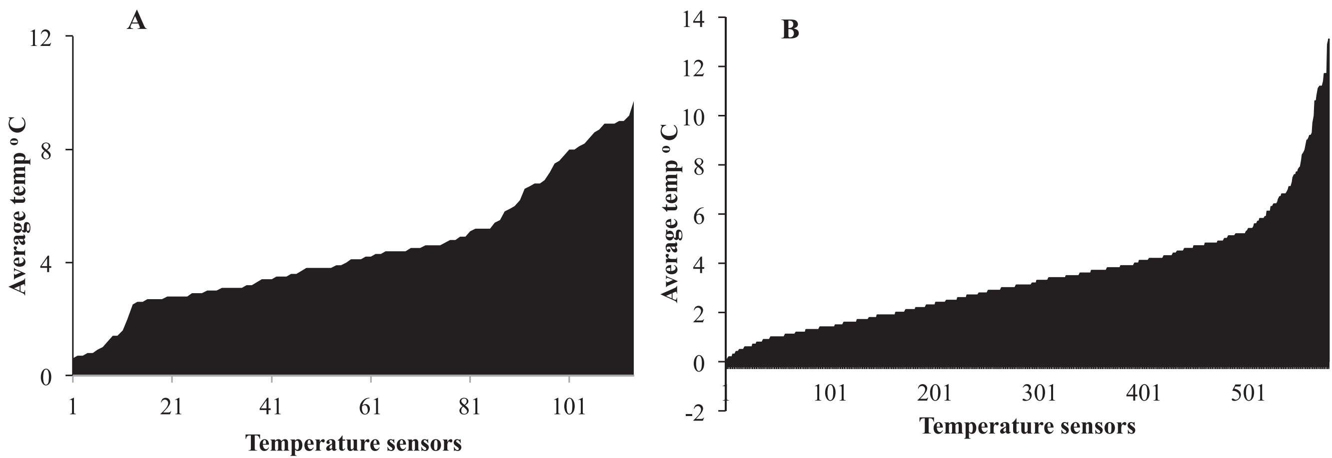


FIGURE 1. *Average temperatures for all temperature sensors during (A) retail storage and (B) retail display.*

4.3, likely because of differences in the level of ripeness among the batches, with an average of 4.1 ± 0.2.

**Temperature profiles of sensors and construction of scenarios.** The average temperatures recorded by all sensors during retail storage and display are shown in Figure 1A and 1B. The 100th, 95th, and 90th percentile average temperatures were 9.7, 8.9, and 8.1°C, respectively, for retail storage and 13.1, 7.8, and 6.1°C, respectively, for retail display. Temperature profiles for the three constructed transport-storage-display scenarios were subsequently developed (Fig. 2). The RMSE and bias between the targeted

FIGURE 2. *Temperature conditions selected for scenarios A, B, and C. The first 2 days represent temperature conditions during transport, the following 4 days represent retail storage, and the last 4 days represent temperature conditions during retail display. Diced products were packaged under high-oxygen atmosphere (AMAP), under passive modified atmosphere (PMAP), and in snap-fit containers that were nonhermetically sealed under ambient air (SN).*

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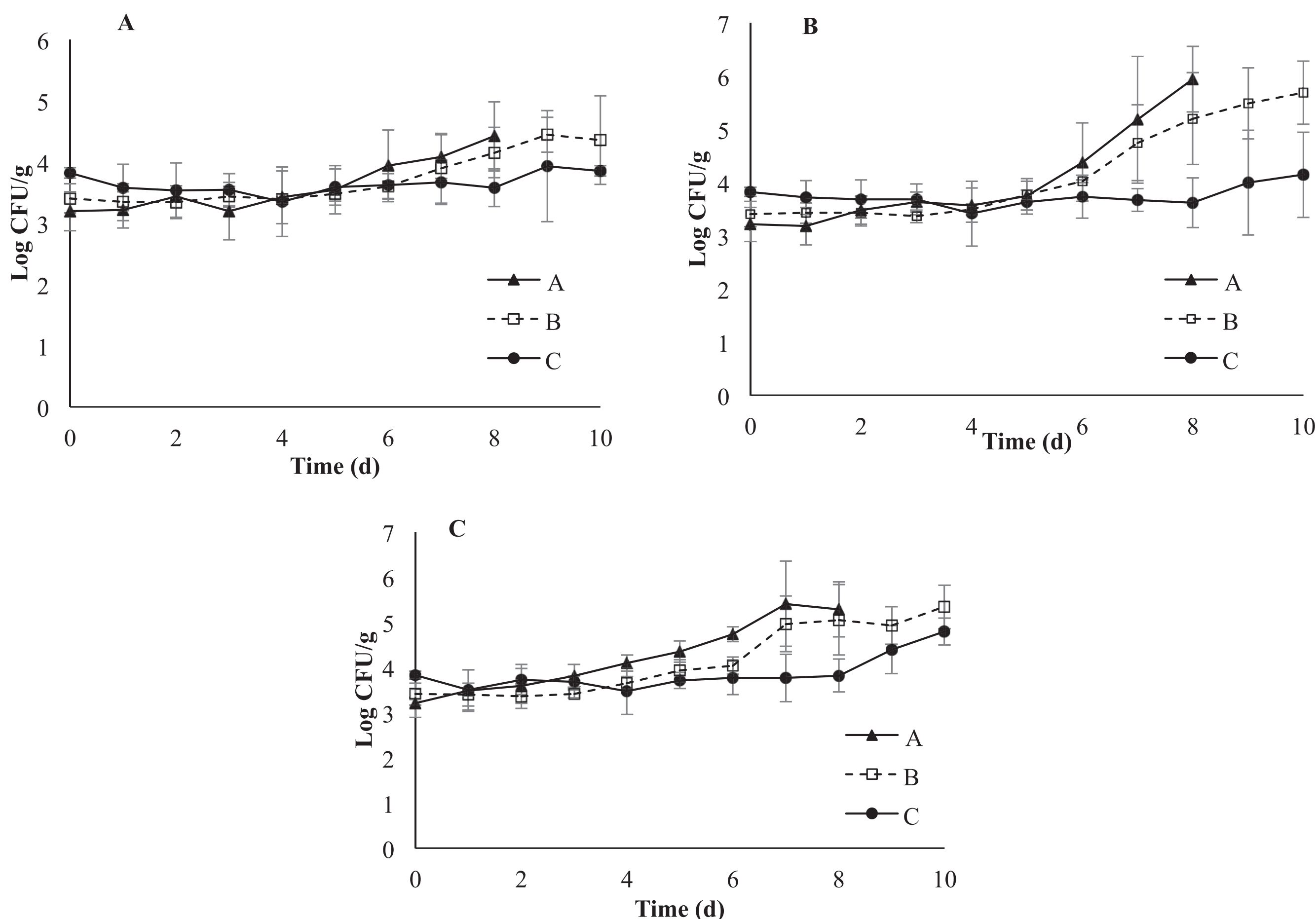


FIGURE 3. *Growth of* L. monocytogenes *in diced onions packaged (A) under high-oxygen atmosphere (AMAP), (B) under passive modified atmosphere (PMAP), and (C) in snap-fit containers that were nonhermetically sealed under ambient air (SN) under temperature conditions of scenarios A, B, and C. Data represent averages from three independent trials, and standard deviations are indicated with error bars.*

and the actual time-temperature profiles were 1.45 and 0.069°C for scenario A, 1.07 and –0.6°C for scenario B, and 1.28 and –0.6°C for scenario C.

**Effect of fluctuating temperatures on pathogen growth in packaged diced product.** Growth curves for *L. monocytogenes* in diced onions and celery and *Salmonella* in diced tomatoes in all packaging systems under the three scenarios are shown in Figures 3 through 5, respectively. Regardless of the packaging system, *Listeria* grew in diced onions under scenario A (Table 1), increasing 1.25 ± 0.47, 2.73 ± 0.45, and 2.32 ± 0.6 log in AMAP, PMAP, and SN-packaged samples, respectively. However, for scenario B, growth of *Listeria* was confined to PMAP and SN packages, with scenario C only supporting growth in SN-packaged diced onions (Table 1). Growth of *L. monocytogenes* was observed in PMAP and SN-packaged diced celery, but not in AMAPs subjected to scenario A (Table 1). Under scenario B, only AMAP diced celery showed a significant increase in numbers of *Listeria,* with no growth seen in packages subjected to scenario C (Table 1). *Salmonella* failed to grow (<1 log CFU/g) in diced tomatoes under any conditions (Table 2), with most packages showing a decline in numbers (Fig. 5). Growth studies conducted under scenario A were discontinued for all products after 8 days because of obvious signs of spoilage, which included visible mold growth, excessive browning, and/or decay.

**Effect of packaging on pathogen growth in diced products under fluctuating temperatures.** Packaging type did not affect ($P > 0.05$) the growth of *L. monocytogenes* in diced onions or celery under time-temperature scenarios B and C (Fig. 6). Under temperature scenario A, growth of *L. monocytogenes* was significantly greater in diced onions ($P = 0.028$) and celery ($P = 0.047$) packaged in PMAP than in AMAP but similar for SN-packaged products ($P > 0.05$). Regardless of the time-temperature scenarios studied, growth of *Salmonella* in diced tomatoes was unaffected by the type of packaging system (Fig. 6).

**Effect of fluctuating temperatures and packaging systems on MAB and YM growth in diced onions, celery, and tomatoes.** The initial MAB and YM counts were approximately 4 log CFU/g after pathogen inoculation. The type of packaging system did not affect MAB or YM growth under any of the three time-temperature scenarios (Fig. 6). Overall, populations of MAB and YM increased

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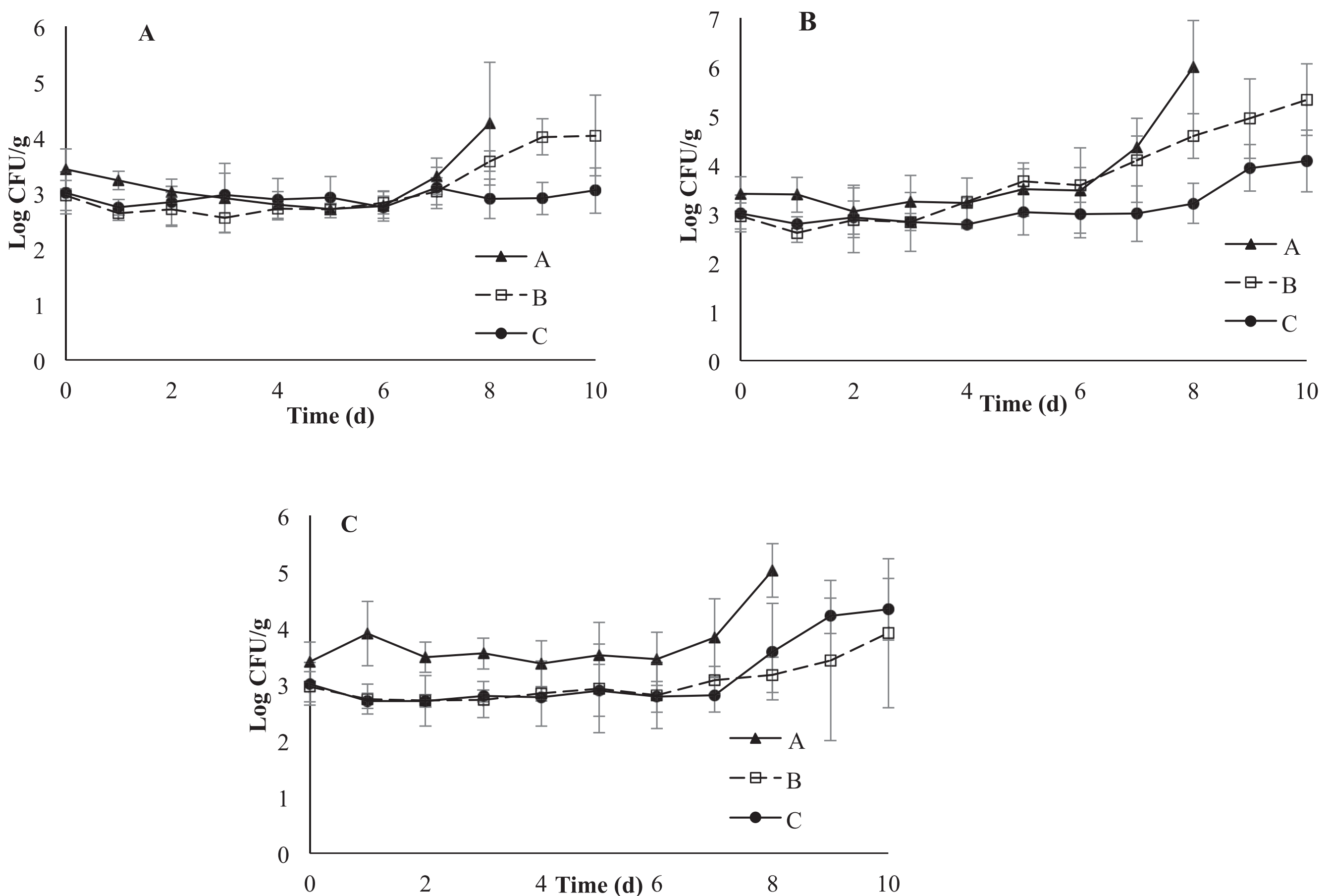


FIGURE 4. *Growth of* L. monocytogenes *in diced celery packaged (A) under high-oxygen atmosphere (AMAP), (B) under passive modified atmosphere (PMAP), and (C) in snap-fit containers that were nonhermetically sealed under ambient air (SN) under temperature conditions of scenarios A, B, and C. Data represent averages from three trials, and standard deviations are indicated with error bars.*

significantly ($P < 0.05$) in all products during storage under the three scenarios. The only exceptions were for AMAP diced onions under scenario B and AMAP diced celery under scenario C, in which YM populations did not increase significantly (Tables 1 and 2).

**Effect of fluctuating temperature on in-package gas composition.** For all products and temperature scenarios, the partial pressure of $O_2$ in PMAPs immediately after sealing steadily decreased from about 21 kPa during the first 3 days of storage, equilibrating to 0.33 ± 0.01 kPa at 5 days (Supplemental Figs. S1, S2, and S3). In PMAP onions, the partial pressure of $CO_2$ increased from approximately 0.38 kPa immediately after sealing to 12.78 ± 0.28, 11.03 ± 0.14, and 10.07 ± 0.25 kPa at 10 days under temperature scenarios A, B, and C, respectively (Fig. S1). The partial pressure of $CO_2$ in PMAP diced celery increased from 0.38 kPa immediately after sealing to 11.62 ± 0.76, 11.19 ± 0.01, and 10.14 ± 0.13 kPa at 10 days under temperature scenarios A, B, and C, respectively (Fig. S2). Similarly, the partial pressure of $CO_2$ in PMAP tomatoes increased from 0.38 kPa after sealing to 12.26 ± 0.28, 10.24 ± 1.45, and 10.21 ± 0.01 kPa at 10 days under temperature scenarios A, B, and C, respectively (Figs. S1, S2, and S3). For all PMAP products except celery at 10 days, the partial pressure of $CO_2$ in products stored under scenario A was significantly higher ($P < 0.05$) than under scenarios B and C throughout storage (Figs. S1, S2, and S3).

Immediately after sealing, partial pressures of $O_2$ and $CO_2$ in the AMAPs were about 94 and 0.5 kPa, respectively. Unlike PMAPs, $O_2$ and $CO_2$ in AMAPs did not reach equilibrium for any of the three products or temperature scenarios. For scenarios A and B, the $O_2$ partial pressure rapidly decreased to about 27, 13, and 19 kPa for diced onions, celery, and tomatoes, respectively, after 5 days of storage, except for AMAP onions under scenario B, for which the $O_2$ partial pressure decreased to about 36 kPa. Some fluctuations in $CO_2$ concentration were observed in AMAPs, especially under scenario A, but these fluctuations were independent of the three scenarios. The $CO_2$ partial pressure for scenario A generally increased from about 0.5 to 13 kPa after 10 days compared with about 11 and 10 kPa for scenarios B and C, respectively (Figs. S1, S2, and S3).

**Growth model and parameter estimation.** The primary and secondary model parameters were estimated based on growth data under constant temperature (Fig. S4). Overall accuracy of the fittings (i.e., RMSE) was 0.64 and 0.90 log CFU/g for diced onion and celery, respectively (Table 3). Although 30% more accurate for onions than celery, both models differed by more than 1 log CFU/g. These models were also validated using the growth data

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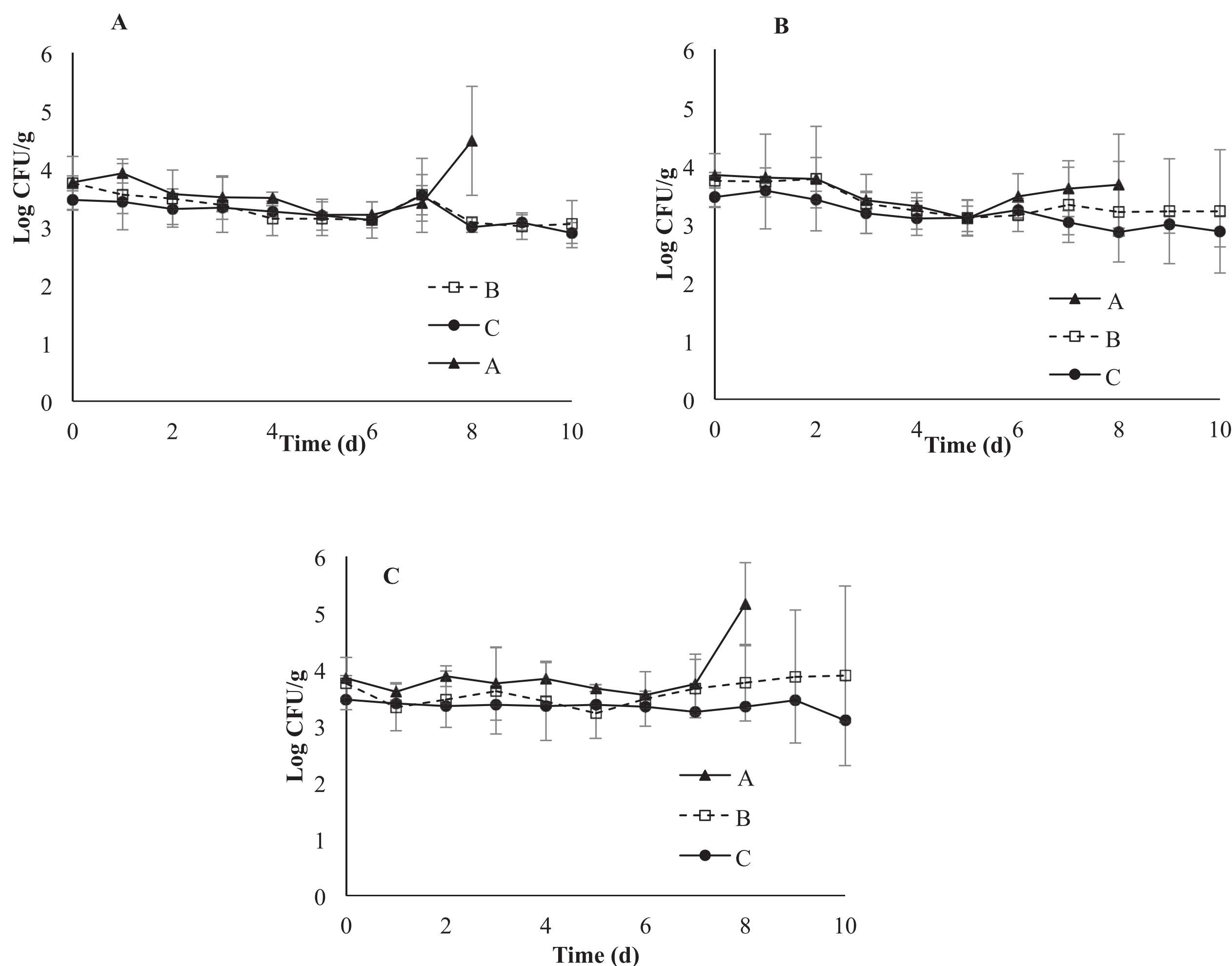


FIGURE 5. *Growth of* Salmonella *in diced tomatoes packaged (A) under high-oxygen atmosphere (AMAP), (B) under passive modified atmosphere (PMAP), and (C) in snap-fit containers that were nonhermetically sealed under ambient air (SN) under temperature conditions of scenarios A, B, and C. Data represent averages from three independent trials, and standard deviations are indicated with error bars.*

obtained under fluctuating temperatures (Fig. 7), with accuracy assessed in terms of RMSE, bias, and APZ (Table 4). For diced onion, RMSE of the prediction ranged from 0.42 to 1.53, with a negative bias (overprediction) seen for temperature scenario A. Based on RMSE, greater accuracy of the prediction was seen for diced celery compared with onion. The celery prediction biases were all positive, ranging from 0.03 for temperature scenario A to 0.19 for scenario B. In terms of APZ, the model was a better predictor of *Listeria* growth in diced celery than onion, with at least 82% of the predictions within the acceptable PEs (Table 4).

## DISCUSSION

Maintaining refrigeration temperatures during commercial storage and distribution is crucial for minimizing microbial growth in fresh-cut produce. Although complete avoidance of temperature fluctuations during commercial handling of produce is unrealistic, effort must be made to reduce prolonged exposure to higher temperatures. Based on the time-temperature profiles obtained during transport *(39)*, retail storage, and display *(8)*, temperatures were highest during retail display, particularly during summer. These observations are consistent with the findings of Nunes et al. *(26)*, who reported a temperature peak of 19.2°C in display blocks.

The low RMSE and bias values indicated good simulation of the real-time–temperature profiles under laboratory conditions. The three scenarios were chosen to reflect multiple levels of temperature abuse that may occur during postprocess handling of fresh-cut produce. Scenario A, which reflected the 100th percentile average temperature data during retail storage and display, tended to support higher pathogen growth, followed by scenarios B and C under a given packaging system. However, obvious signs of spoilage (browning of celery and sliming of onions and tomatoes) were evident for all scenario A products, irrespective of the packaging system. Although spoilage will reduce the food safety burden, because such products are less likely to be consumed, the economic impact of food spoilage is significant. High produce display temperatures at three local retailers in Florida accounted for about 55% of total product waste over a 6-week period *(26)*.

No products stored under scenario B showed signs of spoilage after 10 days. However, the population of *L.*

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TABLE 1. *Changes in the population of* L. monocytogenes, *mesophilic aerobic bacteria (MAB), and yeast and mold (YM) in diced onions and celery during storage under the fluctuating temperature conditions of the 100th (A), 95th (B), and 90th (C) percentile average profiles*[a]

| Sample | Packaging | Scenario | *L. monocytogenes* | MAB | YM |
|---|---|---|---|---|---|
| Onions | AMAP | A | 1.25 ± 0.47* | 2.31 ± 1.21* | 3.12 ± 1.18* |
| | | B | 1.18 ± 0.74 | 3.14 ± 1.85* | 3.23 ± 2.29 |
| | | C | 0.47 ± 0.47 | 4.17 ± 1.05* | 4.34 ± 0.17* |
| | PMAP | A | 2.73 ± 0.45* | 2.76 ± 1.04* | 2.59 ± 0.3* |
| | | B | 2.27 ± 0.79* | 2.74 ± 0.8* | 2.89 ± 1.03* |
| | | C | 0.49 ± 0.55 | 4.70 ± 0.71* | 5.01 ± 0.47* |
| | SN | A | 2.32 ± 0.6* | 3.15 ± 1.83* | 3.27 ± 1.62* |
| | | B | 2.01 ± 0.68* | 4.46 ± 0.42* | 4.59 ± 0.43* |
| | | C | 1.11 ± 0.14* | 4.87 ± 0.47* | 4.26 ± 0.07* |
| Celery | AMAP | A | 0.86 ± 0.74 | 2.85 ± 0.31* | 3.20 ± 0.16* |
| | | B | 1.45 ± 0.08* | 3.75 ± 1.04* | 3.50 ± 0.67* |
| | | C | 0.15 ± 0.19 | 4.12 ± 1.68* | 3 ± 1.41 |
| | PMAP | A | 2.59 ± 0.89* | 3.24 ± 1.19* | 3.86 ± 0.58* |
| | | B | 2.37 ± 0.97 | 3.94 ± 0.4* | 3.72 ± 0.42* |
| | | C | 1.13 ± 0.71 | 4.28 ± 1.40* | 3.29 ± 0.94* |
| | SN | A | 1.62 ± 0.22* | 3.05 ± 0.9* | 3.29 ± 1.38* |
| | | B | 1.05 ± 1.01 | 4.73 ± 0.45* | 4.37 ± 0.32* |
| | | C | 1.25 ± 0.54 | 4.57 ± 1.35* | 3.93 ± 0.89* |

[a] Diced products were packaged under high-oxygen atmosphere (AMAP), under passive modified atmosphere (PMAP), or in snap-fit containers that were nonhermetically sealed under ambient air (SN). Values are presented in log CFU per gram and represent the means ± standard deviations of maximum growth ($N_{max} - N_o$) of three independent trials. $N_o$ is the population at inoculation, and $N_{max}$ is the maximum population reached during storage. * indicates significant increase in bacterial population ($P < 0.05$).

TABLE 2. *Changes in the population of* Salmonella, *mesophilic aerobic bacteria (MAB), and yeast and mold (YM) in diced tomatoes during storage under the fluctuating temperature conditions of the 100th (A), 95th (B), and 90th (C) percentile average profiles*[a]

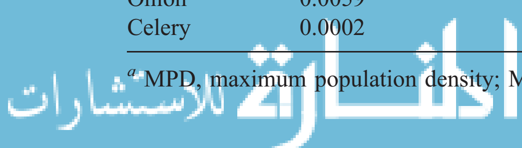
| Sample | Packaging | Scenario | *Salmonella* | MAB | YM |
|---|---|---|---|---|---|
| Tomatoes | AMAP | A | 0.19 ± 0.04 | 3.32 ± 1.19* | 4.01 ± 0.65* |
| | | B | 0.23 ± 0.4 | 4.32 ± 1.08* | 4.09 ± 0.84* |
| | | C | 0.37 ± 0.38 | 2.96 ± 1.53* | 3.27 ± 0.93* |
| | PMAP | A | 0.36 ± 0.37 | 3.24 ± 0.51* | 3.59 ± 1.10* |
| | | B | 0.31 ± 0.32 | 3.93 ± 1.23* | 4.13 ± 1.39* |
| | | C | 0.15 ± 0.22 | 3.63 ± 1.18* | 3.07 ± 0.68* |
| | SN | A | 1.30 ± 0.78 | 3.86 ± 0.33* | 4.27 ± 0.35* |
| | | B | 0.42 ± 0.44 | 4.50 ± 1.26* | 4.24 ± 0.98* |
| | | C | 0.23 ± 0.25 | 4.58 ± 0.77* | 4.11 ± 0.21* |

[a] Diced products were packaged under high-oxygen atmosphere (AMAP), under passive modified atmosphere (PMAP), or in snap-fit containers that were nonhermetically sealed under ambient air (SN). Values are presented in log CFU per gram and represent the means ± standard deviations of maximum growth ($N_{max} - N_o$) of three independent trials. $N_o$ is the population at inoculation, and $N_{max}$ is the maximum population reached during storage. * indicates significant increase in bacterial population ($P < 0.05$).

TABLE 3. *Estimated parameters of Baranyi primary model with secondary models for predicting the growth of* L. monocytogenes *in packaged diced onion and celery under constant temperature (12, 16, and 23°C)*[a]

| | $\mu_{max}$ (maximum specific growth rate) | | $y_{max}$ (MPD) | | Physiological state | Shape factors | | Accuracy |
|---|---|---|---|---|---|---|---|---|
| Product | *b* | Min | $\beta_0$ | $\beta_1$ | $h_0$ | *m* | *n* | RMSE |
| Onion | 0.0059 | 10.14 | 7.5005 | 0.0586 | 2.5133 | 1 | 0.1034 | 0.64 |
| Celery | 0.0002 | 0.00 | 8.2796 | 0.0592 | 0.9910 | 1 | 0.0111 | 0.90 |

[a] MPD, maximum population density; Min, minimum; RMSE, root mean square error.



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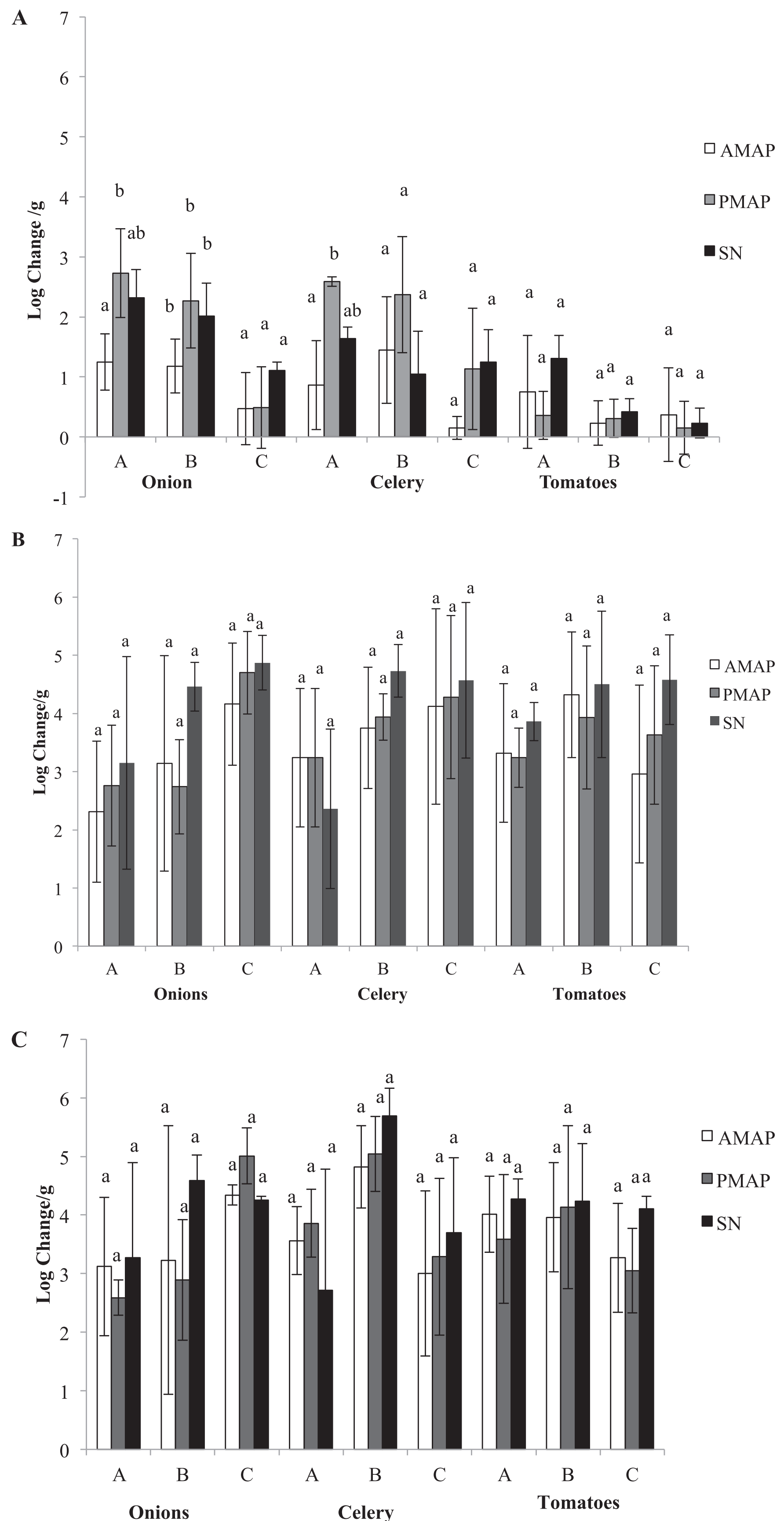


FIGURE 6. *Maximum changes in the (A) population of* L. monocytogenes *in diced onion and celery and* Salmonella *in diced tomatoes and populations of (B) mesophilic aerobic bacteria and (C) yeast and mold in diced onions, celery, and tomatoes. Diced products were packaged (A) under high-oxygen atmosphere (AMAP), (B) under passive modified atmosphere (PMAP), and (C) in snap-fit containers that were nonhermetically sealed under ambient air (SN) under temperature conditions of scenarios A, B, and C. Data represent averages from three independent trials, and standard deviations are indicated with error bars. Different letters on the same profile and diced product indicate significant differences (*$P < 0.05$*) in growth among the three packaging systems.*

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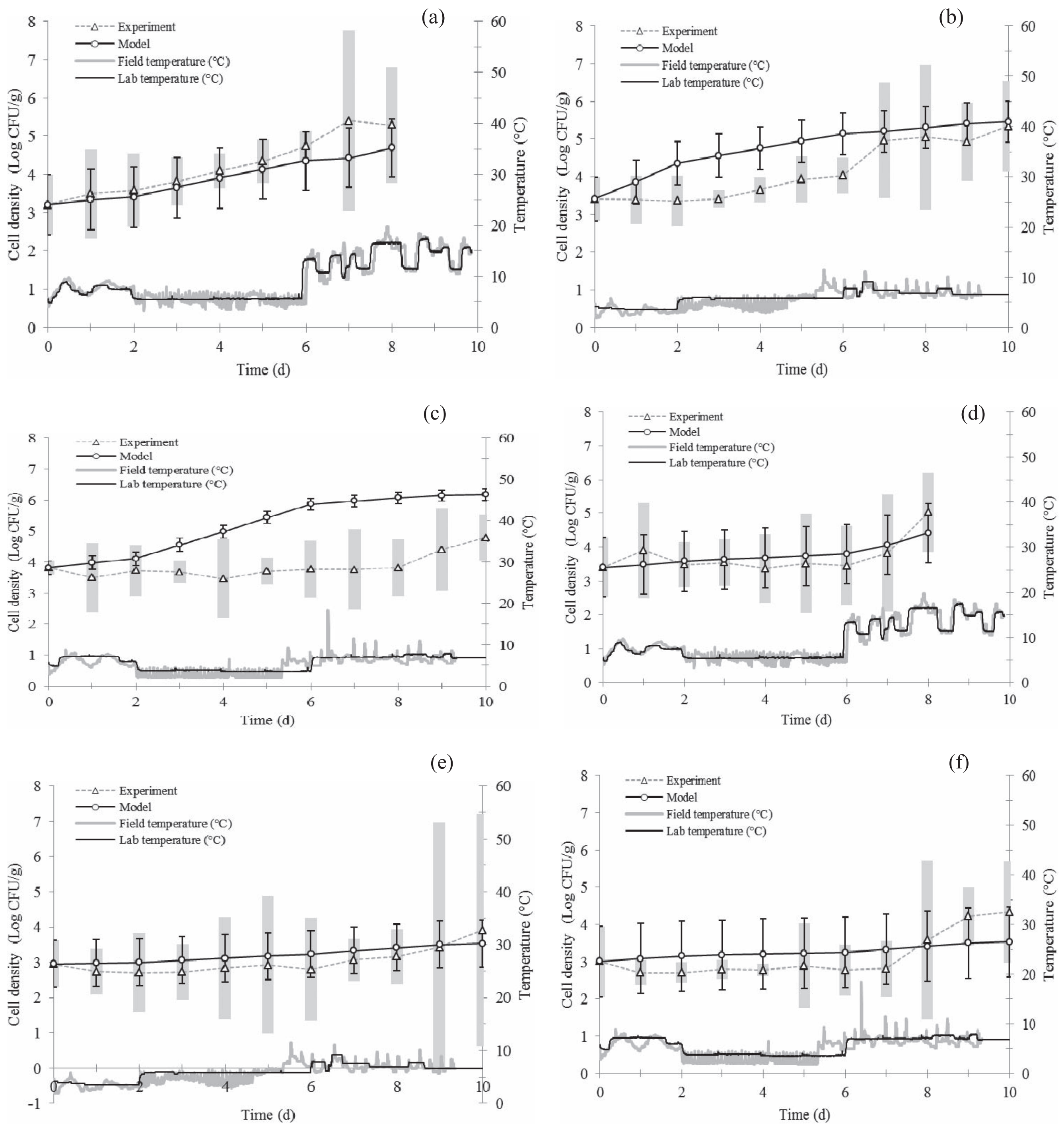


FIGURE 7. *Validation of the model prediction (95% confidence interval) for* L. monocytogenes *growth in packaged diced onion (a, b, and c) and celery (d, e, and f) products subjected to three temperature histories based on actual transportation–retail temperature histories (A for a and d, B for b and e, and C for c and f) for up to 10 days. The shaded bar represents the 95% confidence interval of the measured growth.*

*monocytogenes* in PMAP and SN-packaged diced onions and in PMAP diced celery stored under scenario B increased more than 2 log CFU/g during storage. Scenario C, consisting of profiles from sensors with 90th percentile average temperatures, supported less pathogen growth compared with scenarios A and B. The capacity of different strains of *Salmonella* to grow in tomatoes has been characterized *(3)*. However, none of the temperature conditions considered in this study supported significant *Salmonella* growth in diced tomatoes. *Salmonella* may have been unable to grow under the conditions tested because of initial cold stress (<8°C) during 2 days of simulated transport, with subsequent growth impaired as the temperature increased during simulated retail storage and display. This hypothesis is supported by the findings of Daş et al. *(15),* who observed a significant reduction in the population of *Salmonella* Enteritidis on spot-inoculated cherry tomatoes packaged under passive modified atmosphere during 10

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TABLE 4. *RMSE, bias, and APZ between predicted and observed growth data of* L. monocytogenes *in packaged diced onion and celery under the dynamic temperature profile based on actual transport, retail storage, and retail display data*[a]

| Product | Temp profiles | RMSE | Bias | APZ (%) |
|---|---|---|---|---|
| Onion | A | 0.42 | −0.32 | 78 |
| | B | 0.76 | 0.63 | 64 |
| | C | 1.53 | 1.33 | 36 |
| Celery | A | 0.31 | 0.03 | 89 |
| | B | 0.28 | 0.19 | 100 |
| | C | 0.47 | 0.12 | 82 |

[a] RMSE, root mean square error; APZ, acceptable prediction zone.

days of storage at 7°C. Produce spoilage results from the combined effects of microbial growth, enzymatic activity, and physiological change. Populations of MAB and YM in produce may serve as indices of spoilage *(18)*. Regardless of the packaging system used, all products under scenario A were visually spoiled after 8 days of storage. In contrast, all scenario B and C products remained visually unspoiled after 10 days of storage regardless of packaging system, although the maximum increase in MAB and YM populations was similar for three scenarios regardless of the packaging system (Fig. 6).

Modified atmosphere packaging is widely used to retard spoilage processes in fresh-cut produce. However, the anaerobic conditions that can occur in PMAPs have raised safety concerns *(16, 27)*. The $O_2$ partial pressures in PMAP diced onions, tomatoes, and celery reached a minimum of 0.33, 0.38, and 0.35 kPa, respectively, after 3 days of storage under scenario A, while similar concentrations were observed after 5 days for scenarios B and C. Despite the modified atmosphere in PMAPs, *L. monocytogenes* growth in PMAP and SN-packaged diced products was similar regardless of the storage conditions (Fig. 6). Most studies have reported that *L. monocytogenes* growth is unaffected by low $O_2$ partial pressure. Jacxsens et al. *(19)* observed no difference in the growth of *L. monocytogenes* at 7°C when shredded chicory, endive, and shredded iceberg lettuce were packaged under an equilibrium atmosphere of 2 to 3% $O_2$, 2 to 3% $CO_2$, and 94 to 96% $N_2$, compared with normal atmospheric levels of $O_2$, $CO_2$, and $N_2$. Similar growth of *E. coli* O157:H7 and *Salmonella* has been reported for fresh-cut apples packaged in ambient air or a passively modified atmosphere *(1)*.

Under scenario A, *L. monocytogenes* populations were significantly lower in diced onions and celery packaged under a high-oxygen atmosphere ($P < 0.05$) compared with PMAP. The inhibitory effect of high oxygen levels against *L. monocytogenes* and other foodborne pathogens is well characterized. In one study, a high-oxygen atmosphere of 95 kPa inhibited *L. monocytogenes* in packaged fresh-cut celery held at 7°C *(17)*. In other work, *L. monocytogenes* and *Salmonella* Typhimurium generally failed to grow on various minimally processed vegetables when packaged in a 90-kPa oxygen atmosphere and stored at 8°C, although the lag phase for *L. monocytogenes* was extended *(2)*. However, passive modified atmosphere packaging can reportedly enhance the growth of *L. monocytogenes* on lettuce, coleslaw mix, and soybean sprouts.

The bactericidal effect of high oxygen levels may be because of auto-oxidation of cytochromes in the presence of $O_2$, oxidation of certain enzymes, especially those with sulfhydryl groups or disulfide bridges, accumulation of injurious reactive $O_2$ species, lipid peroxidation, and formation of superoxide radicals ($O_2^-$) *(20)*. Moreover, enhanced genotoxicity of the reaction by-products from ferrous iron and oxygen at high oxygen concentrations is well documented *(14, 33)*. Although most bacteria have evolved defense mechanisms against oxidative stress using OxyR and SoxRS transcriptional regulators, there may be an energy tradeoff between genome maintenance and cell proliferation *(10)*.

Although high-oxygen atmospheres may inhibit some microorganisms in fresh-cut produce, deteriorative processes such as respiration and enzymatic activity may be enhanced. Accelerated respiration rates may have contributed to spoilage of diced onions, celery, and tomatoes after about 3 days of storage in the high-oxygen atmosphere packages, regardless of temperature scenario. Fresh-cut produce is more susceptible to rapid respiration than intact produce because of extensive tissue damage resulting from slicing and dicing. In addition, the higher juice levels observed when diced tomatoes were packaged in high-oxygen atmospheres could have resulted from increased respiration and enzyme activity, leading to tissue degradation, and the observed color loss in diced tomatoes and celery was most likely the result of pigment oxidation. Gonzalez-Buesa et al. *(17)* also reported intense yellowing of celery sticks packaged under high-oxygen atmospheres and stored at 7°C.

Overall, the Baranyi primary model coupled with the secondary models and parameters were able to predict *L. monocytogenes* growth in packaged diced onion and celery under dynamic temperature conditions. Koseki and Isobe *(22)* also reported that combined use of the Baranyi primary growth model with the Ratkowsky secondary model and MPD equation reliably predicted the growth of *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* in iceberg lettuce under dynamic temperature conditions. Although the Koseki-Isobe model overestimated *E. coli* O157:H7 and *Salmonella* growth on lettuce, the prediction for *L. monocytogenes* agreed with observed growth data *(22)*. Moreover, Zeng et al. *(39)* used the Koseki-Isobe model to reliably predicated the growth of *L. monocytogenes* and *E. coli* O157:H7 in fresh-cut romaine mix held at fluctuating temperatures. Although some portions of the dynamic temperature histories (Fig. 7) were outside the range of data used to estimate the secondary model for growth rate (12 to 23°C), the three-point estimation (12, 16, and 23°C) with linearity assumption would not be expected to yield significant problems in the extrapolated region (<12°C). In addition, the lower temperatures were not the focus of this study (i.e., temperature abuse scenarios, >10 to 15°C), because most transport and retail display temperatures are maintained below 10 to 15°C. Therefore, the overall modeling approach described in the current

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study can be used to assess current practices associated with transportation, retail storage, and display of various fresh-cut products.

## ACKNOWLEDGMENT

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## SUPPLEMENTAL MATERIAL

Supplemental material associated with this article can be found online at: https://doi.org/10.4315/0362-028X.JFP-18-277.s1.

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