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Fate of *Listeria monocytogenes* in Fresh Apples and Caramel Apples

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ABSTRACT

An outbreak of listeriosis in late 2014 and early 2015 associated with caramel apples led to questions about how this product became a vector for *Listeria monocytogenes*. This investigation aimed to determine information about the survival and growth of *L. monocytogenes* in both fresh apples and caramel apples, specifically examining the effects of site and level of inoculation, inoculum drying conditions, and storage temperature. At a high inoculation level (7 log CFU per apple), *L. monocytogenes* inoculated at the stem end proliferated on Gala caramel apples at both 5 and 25°C and on Granny Smith caramel apples at 25°C by as much as 3 to 5 log CFU per apple. Fresh apples and caramel apples inoculated at the equatorial surface supported survival but not growth of the pathogen. Growth rates (μ_{max}) for apples inoculated at the stem end, as determined using the Baranyi and Roberts growth model, were 1.64 ± 0.27 and 1.38 ± 0.20 log CFU per apple per day for Gala and Granny Smith caramel apples, respectively, stored at 25°C. At a low inoculation level (3 log CFU per apple), *L. monocytogenes* inoculated at the stem end and the equatorial surface survived but did not grow on fresh Gala and Granny Smith apples stored at 25°C for 49 days; however, on caramel apples inoculated at the stem end, *L. monocytogenes* had significant growth under the same conditions. Although certain conditions did not support growth, the pathogen was always detectable by enrichment culture. The inoculation procedure had a significant effect on results; when the inoculum was allowed to dry for 24 h at 5°C, growth was significantly slowed compared with inoculum allowed to dry for 2 h at 25°C. Variation in stick materials did affect *L. monocytogenes* survival, but these differences were diminished once sticks were placed into caramel apples.

Key words: Caramel apples; Fresh apples; Growth kinetics; *Listeria monocytogenes*; Survival

Listeria monocytogenes has caused outbreaks of listeriosis that have been associated with consumption of meats, dairy products, and fresh vegetables, but few documented cases of listeriosis have been linked to fresh fruits. In the United States, *L. monocytogenes* was first involved in an outbreak associated with fresh fruit, specifically cantaloupe, in 2011 *(9)*. A total of 147 illnesses, 142 hospitalizations, and 33 deaths were attributed to this outbreak. Another unusual fruit-linked outbreak of listeriosis occurred in late 2014 and early 2015, and the vector was commercially produced prepackaged caramel apples. This outbreak resulted in a total of 35 illnesses in 12 states and included 34 hospitalizations and seven deaths; the Public Health Agency of Canada also reported one associated case *(10)*. Of the illnesses, 11 were pregnancy related, 1 of which resulted in fetal loss. Of the 31 ill individuals interviewed, 28 reported eating commercially produced prepackaged caramel apples before becoming ill *(10)*. The other three individuals who did not report eating caramel apples did report consuming sliced or whole apples. Caramel apples from three manufacturers were implicated, and further investigation led to one apple grower-packer as the source of the apples. Fresh Gala and Granny Smith apples from the apple grower-packer were shipped either to retailers for direct consumption or to manufacturers to be processed into caramel apples. Only caramel apples, not fresh apples, were associated with the outbreak. The pathogen was isolated from environmental swab samples taken in the storage room and from food contact surfaces at the apple grower-packer facility. Commercial apple contamination by *L. monocytogenes* resulted in recalls of packaged fresh-cut apples in 2001 *(18, 25)* and of packaged apple slices in 2015 *(8)*, although no illnesses were associated with either recall.

How the caramel apples became a vector for the listeriosis outbreak is not known. Apples are not an adequate medium for proliferation of this pathogen because of their low pH (<4.0) *(5)*. *L. monocytogenes* cannot penetrate into the flesh through the peel unless scars or cuts are already present on the apple surface *(4)*. Application of the hot molten caramel during the manufacture of caramel apples provides a thermal impediment to bacterial survival.

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TABLE 1. L. monocytogenes *populations on inoculated fresh apples (without sticks) stored at 5 or 25°C*[a]

Temp (°C)	Apple variety	Inoculation location	Mean ± SD log CFU/apple at[b]: 0 days	1 day	2 days	6 days	9 days	15 days
5	Gala	Stem end	5.7 ± 0.6	5.4 ± 0.8	6.1 ± 0.7	4.4 ± 0.8	5.9 ± 1.0	5.5 ± 0.6
		Surface	2.7 ± 0.3	2.7 ± 0.3	BE[c]	BE	4.7 ± 0.8	BE
25	Gala	Stem end	5.7 ± 0.6	5.4 ± 0.7	5.8 ± 0.7	3.8 ± 0.7	3.9 ± 0.7	3.9 ± 0.5
		Surface	2.7 ± 0.3	BE	BE	BE	5.7 ± 1.5	BE
25	Granny Smith	Stem end	6.7 ± 1.1	5.2 ± 0.9	BE	6.9 ± 1.7	6.0 ± 1.5	2.9 ± 0.5
		Surface	4.8 ± 0.8	BE	BE	BE	5.1 ± 0.8	3.3 ± 0.6

[a] Initial inoculation was 6.9 ± 0.6 log CFU per apple.
[b] Values are means for $n = 6$.
[c] BE, below sensitivity of place count assay (2.5 log CFU per apple). In all cases, *L. monocytogenes* was detectable by enrichment culture.

Current practices for caramel apple production may involve selection of apples based on the manufacturer's specifications, cleaning of apples using water washing, brushing, and sanitizing, dipping and coating the apples with molten caramel, drying, and packaging of the completed caramel apples. After packaging, the finished products may or may not enter the cold chain during transport to retailers, where they are stored at ambient temperature awaiting consumer purchase.

This study was conducted to determine potential factors in caramel apple production that may have influenced the survivability and growth of *L. monocytogenes*. Factors included contamination level, site of contamination, apple variety, storage temperature, presence or absence of caramel coating, and stick material.

MATERIALS AND METHODS

***L. monocytogenes* strains and culture conditions.** Three clinical outbreak isolates of *L. monocytogenes* (573-035, 576-043, and 580-060) from patients with listeriosis associated with the 2014 caramel apple outbreak were kindly provided by the Wisconsin State Laboratory of Hygiene (Madison, WI). All strains were serotype 4b with GX6A16.0012 pulsed-field gel electrophoresis *Asc*I patterns *(24)*. All strains were grown separately in brain heart infusion (BD, Sparks, MD) broth at 37°C for 16 to 18 h with shaking at 200 rpm.

Apple selection and experimental design. Whole fresh waxed Gala apples and Granny Smith apples were purchased from local retail supermarkets. Apples with obvious bruising or cuts were discarded. Average weights of apples used for experiments were 178.9 ± 7.2 and 177.0 ± 10.1 g for Gala and Granny Smith apples, respectively. Experimental variables included temperature during storage (5 and 25°C), inoculation level (10^7 or 10^3 CFU per apple), inoculation site (equatorial surface and stem end), inoculum drying conditions (5°C for 24 h or 25°C for 2 h), and caramel coating with wood stick insertion. Apples were prepared in triplicate for each variable for each timepoint of 0, 1, 2, 6, 9, and 15 days for Gala apples stored at 5 and 25°C and Granny Smith apples stored at 25°C and for each timepoint of 0, 7, 14, 21, 28, 35, 42, and 49 days for Gala and Granny Smith apples stored at 25°C. For 5°C storage studies, variables were stem end inoculation, equatorial surface inoculation, stem end inoculation with caramel coating and stick, and equatorial surface inoculation with caramel coating and stick. For 25°C storage studies, an additional variable was stick material (plastic, paper, or wood). For each caramel apple experiment, uninoculated control apples consisting of caramel coating and stick were assayed for pH and spoilage. All experiments were conducted in two independent trials.

***L. monocytogenes* inoculation of apples.** Overnight cultures of *L. monocytogenes* strains were normalized, washed with Butterfield's phosphate buffer (BPB; pH 7.4), and combined equally to make a cocktail of approximately 9 or 5 log CFU/ml. Apples were inoculated at the stem end or along the equatorial surface by pipetting 10 μl of the *L. monocytogenes* cocktail to yield final levels of 6.9 ± 0.6 or 3.1 ± 0.2 log CFU per apple, as determined by plate count assay of the cocktail on PALCAM (BD) agar. The inoculum was dried for 2 h at 25°C or for 24 h at 5°C. The *L. monocytogenes* population recovered from apples after drying and with or without caramel was approximately 2 log CFU lower than the initial inoculum (data not shown).

Preparation of caramel apples. A wood stick typically used for making caramel apples was inserted approximately 3 to 4 cm into the stem end of each apple prior to caramel coating. Where indicated for some experiments, paper or plastic sticks also were used. Caramel pieces (containing corn syrup, sugar, milk, fructose, hydrogenated coconut oil, butter, mono- and diglycerides, salt, soy lecithin, and vanillin; inherent water activity of 0.66) were purchased from local retailers and melted to 76°C in a 4211c Twin Caramel Apple Dip Warmer according to the manufacturer's instructions (Gold Medal Products Co., Bensenville, IL). Temperature was monitored with a candy thermometer inserted into the caramel. For apples on which inoculum had dried at 5°C for 24 h, apples were equilibrated to room temperature prior to dipping. Apples were dipped manually into the caramel so that approximately 3 cm of the stick was covered. Excess caramel was allowed to drip off, and the apples were placed onto wax paper to dry at ambient temperature for 2 h. After drying, all apples, with or without caramel, were placed into food-grade clamshell containers for storage at 5 or 25°C for various time periods. Fresh apple and caramel apple trials were conducted concurrently.

Enumeration of *L. monocytogenes* from apples. At the appropriate time intervals, apples were taken out of clamshells and placed into 3-liter stomacher bags. Visual and odor changes in apples were recorded. Apples were smashed five to seven times with a rubber mallet, 350 ml of buffered *Listeria* enrichment broth (BLEB, BD) was added, and the mixture was stomached for 1 min at 180 rpm in a stomacher (model 3500, Seward Laboratory Systems Inc., Davie, FL). BLEB was chosen because of its superior capacity to neutralize the acid from the apples and maintain the pH at approximately 7.0. A 10-ml sample of the

TABLE 2. L. monocytogenes *populations and growth kinetics on inoculated caramel apples stored at 5 or 25°C*[a]

Temp (°C)	Apple variety	Inoculation location	Mean ± SD log CFU/apple at:						Growth kinetics[b]		
			0 days	1 day	2 days	6 days	9 days	15 days	μ_{max} ± SE	r^2	Time to 1-log increase (h)
5	Gala	Stem end	5.4 ± 0.8	5.7 ± 0.8	7.0 ± 1.0	8.0 ± 1.3	8.4 ± 1.7	8.5 ± 2.2	0.95 ± 0.21 A	0.80	26.2 ± 0.2
		Stem end, dried[c]	3.3 ± 0.4	2.9 ± 0.3	BE[d]	3.5 ± 0.4	6.5 ± 1.3	6.4 ± 1.6	0.80 ± 0.31 A	0.81	172.8 ± 0.6
		Surface	4.7 ± 0.6	4.8 ± 0.6	4.5 ± 0.8	BE	4.4 ± 0.8	BE	ND[e]	ND	ND
25	Gala	Stem end	5.4 ± 0.8	5.6 ± 1.3	7.9 ± 2.0	9.5 ± 1.6	10.0 ± 2.5	9.6 ± 1.6	1.64 ± 0.27 B	0.89	14.9 ± 0.1
		Stem end, dried[c]	3.3 ± 0.4	BE	6.8 ± 2.4	7.8 ± 1.3	8.1 ± 1.4	8.6 ± 1.5	0.85 ± 0.19 B	0.85	29.3 ± 0.3
		Surface	4.7 ± 0.6	4.7 ± 0.6	4.4 ± 0.5	4.6 ± 0.5	4.4 ± 0.8	BE	0.38 ± 0.23 B	0.23	287.5 ± 2.3
25	Granny Smith	Stem end	5.5 ± 0.9	7.2 ± 1.2	8.1 ± 1.2	8.6 ± 1.4	8.7 ± 1.4	8.9 ± 1.5	1.38 ± 0.20 C	0.91	17.6 ± 0.2
		Stem end, dried[c]	4.4 ± 0.6	5.2 ± 0.9	6.8 ± 1.1	8.4 ± 1.4	8.5 ± 1.2	8.8 ± 1.5	1.19 ± 0.17 C	0.95	20.4 ± 0.2
		Surface	6.3 ± 1.1	4.6 ± 0.7	BE	BE	6.9 ± 1.2	6.4 ± 1.1	0.15 ± 0.09 C	0.11	219.2 ± 11.6

[a] Initial inoculation was 6.9 ± 0.6 log CFU per apple. Values are means for $n = 6$.
[b] μ_{max} ± SE, mean maximum growth rate (log CFU per apple per day) ± standard error. Means with different letters are significantly different ($P < 0.05$) for comparisons of the same inoculation locations on different apple varieties at both temperatures; r^2, coefficient of determination.
[c] Inoculum placed at the stem end was dried at 5°C for 24 h.
[d] BE, below sensitivity of plate count assay (2.5 log CFU per apple). In all cases, *L. monocytogenes* was detectable by enrichment culture.
[e] ND, not determined.

homogenate was placed into a 15-ml tube. Serial dilutions in BLEB were spread plated in duplicate onto PALCAM agar. For timepoints at which *Listeria* was expected to be below the sensitivity of the plate count assay of 3 log CFU/ml, duplicate 1-ml aliquots of homogenates were plated over three PALCAM agar plates to increase the assay sensitivity to 2.5 log CFU/ml. PALCAM plates were incubated at 37°C for 48 h. All apple homogenates in BLEB were also used for enrichment cultures. These cultures were incubated at 30°C for 4 h, supplements were added, and the cultures were incubated again at 30°C for 24 h. When no growth was present on enumeration plates, the BLEB enrichment cultures were streaked onto PALCAM plates and tested for the presence of *L. monocytogenes* using the *Listeria* Visual Immunoprecipitate Assay (BioControl Systems Inc., Bellevue, WA) according to the manufacturer's directions.

Enumeration of native microbiota from apples. Populations of native microbiota on apples were monitored at each timepoint using the control apples (no inoculation, with caramel coating and stick). Apples were stomached as previously described, and the homogenates were serially diluted and plated in duplicate onto Dichloran Rose Bengal (DRBA) and deMan Rogosa Sharpe (MRS) agars (BD) for enumeration of presumptive yeasts and molds and lactic acid bacteria, respectively. DRBA plates were incubated at 25°C for 48 h, and MRS plates were incubated anaerobically at 37°C for 72 h before enumeration.

Apple pH. Apple pH was monitored at each timepoint using the control apples (no inoculation, with caramel coating and stick). pH was measured using a PH/ORP waterproof pH spear (Oakton Instruments, Vernon Hills, IL) by inserting the tip of the spear into the stem end of the apple and allowing the pH reading to equilibrate for 2 min.

***L. monocytogenes* survival on various stick materials.** Overnight cultures of *L. monocytogenes* strains were normalized, washed with BPB, and combined equally to make a cocktail of approximately 9 log CFU/ml. Wood, paper, and plastic sticks (14 to 15 cm long) were each inoculated with five 2-μl spots, yielding a final inoculation of 7.0 ± 0.7 log CFU per stick. Sticks were stored in sterile containers at 5 or 25°C. At 0, 2, 5, 7, and 15 days, triplicate samples of each type of stick material were placed into sterile 1.2-liter stomacher bags with 100 ml of BLEB, massaged by hand for 1 min, and then stomached at 180 rpm for 1 min. A 10-ml sample of this homogenate was transferred to a 15-ml tube, and serial dilutions in BLEB were plated in duplicate onto PALCAM agar, which were incubated at 37°C for 48 h.

Modeling. The DMFit version 3.0 (Institute of Food Research, Norwich, UK) Excel (Microsoft, Redmond, WA) add-on from ComBase (www.combase.cc) was used to model the maximum growth rates (μ_{max}) and lag phases of the *L. monocytogenes* cocktail based on the Baranyi and Roberts *(3)* model. The value at time 0 was the *L. monocytogenes* recovered from the fresh apples (after inoculum drying) or from the caramel apples (after 2 h of drying of caramel). Calculation of μ_{max} was based on the *L. monocytogenes* recovered at different timepoints relative to time 0. Growth of *L. monocytogenes* on the apples was defined by the calculation of a positive growth rate using DMFit. Survival of the pathogen was defined by the detection of the pathogen after enrichment culture. Linear regression analysis with the μ_{max} values was used to determine the time to achieve 1 log CFU of growth, assuming no lag phase, at each condition and temperature.

TABLE 3. L. monocytogenes *populations on inoculated caramel apples stored long term at 25°C*[a]

Apple variety	Inoculation location	Mean ± SD log CFU/apple at[b]: 0 days	7 days	14 days	21 days	28 days	35 days	42 days	49 days
Gala	Stem end	BE[c]	8.6 ± 1.4	8.8 ± 1.0	9.1 ± 1.8	9.3 ± 1.6	8.8 ± 1.8	8.0 ± 4.0	9.4 ± 3.1
	Stem end, dried[d]	BE	6.7 ± 1.4	8.2 ± 1.1	9.8 ± 1.7	8.3 ± 1.4	7.6 ± 1.3	6.6 ± 3.3	7.5 ± 2.4
	Surface	BE	BE	BE	BE	BE	BE	BE	BE
Granny Smith	Stem end	BE	8.9 ± 1.3	8.5 ± 1.4	9.1 ± 1.5	8.8 ± 1.4	7.5 ± 1.3	8.2 ± 1.0	8.4 ± 1.4
	Stem end, dried[d]	BE	7.9 ± 1.6	8.0 ± 1.0	9.1 ± 1.5	7.7 ± 1.3	7.4 ± 2.5	6.9 ± 3.3	7.7 ± 3.7
	Surface	BE	BE	2.9 ± 0.5	BE	BE	BE	BE	BE

[a] Initial inoculation was 3.1 ± 0.2 log CFU per apple.
[b] Values are means for $n = 6$.
[c] BE, below sensitivity of plate count assay (2.5 log CFU per apple). In all cases, *L. monocytogenes* was detectable by enrichment culture.
[d] Inoculum placed on the stem end was dried at 5°C for 24 h.

Statistical analysis. Data were statistically evaluated using Tukey's adjusted one-way analysis of variance using GraphPad InStat for Windows. A P value of less than 0.05 was considered significant.

RESULTS

***L. monocytogenes* survival on fresh apples.** At an initial inoculation level of 7 log CFU per apple, *L. monocytogenes* inoculated both at the stem end and on the equatorial surface survived on Gala apples stored at both 5 and 25°C and on Granny Smith apples stored at 25°C; however, populations decreased by approximately 1 to 4 log CFU per apple (Table 1). Although *L. monocytogenes* levels were below the sensitivity of the plate count assay (2.5 log CFU per apple) at various timepoints during storage in these experiments, the pathogen was still present as determined by enrichment culture (data not shown).

At the initial inoculation level of 3 log CFU per apple, *L. monocytogenes* inoculated at the stem end and on the surface did not produce detectable growth on fresh Gala or Granny Smith apples stored at 25°C for 49 days. At most of the timepoints, the population of *L. monocytogenes* was below the sensitivity of the plate count assay. Nevertheless, the pathogen survived on the fresh apples, as determined by enrichment culture (data not shown).

***L. monocytogenes* survival and growth on caramel apples.** At an initial inoculation level of 7 log CFU per apple, *L. monocytogenes* inoculated both at the stem end and on the surface were capable of surviving and at times growing on Gala caramel apples stored at 5 and 25°C and on Granny Smith caramel apples stored at 25°C (Table 2). On Gala caramel apples inoculated at the stem end stored at 5°C, the population of *L. monocytogenes* increased by nearly 3 log CFU after 15 days of incubation. On the surface-inoculated Gala caramel apples stored at 5°C, *L. monocytogenes* remained nearly at initial inoculation levels or decreased to below the sensitivity of the place count assay; however, the presence of the pathogen was detectable by enrichment culture. The highest μ_{max} value for Gala caramel apples stored at 5°C was found for *L. monocytogenes* inoculated at the stem end, 0.95 log CFU per apple per day, leading to a 1-log increase in only 26.18 h (Table 2).

During storage at 25°C, *L. monocytogenes* inoculated at the stem end at 7 log CFU per apple had 3- to 4-log increases in population on both Gala and Granny Smith caramel apples (Table 2). The μ_{max} value was 1.64 log CFU per apple per day on the stem end-inoculated Gala caramel apples, and 1.38 log CFU per apple per day on Granny Smith caramel apples, leading to a 1-log increase in just 17.58 h. Surface-inoculated caramel apples stored at 5 and 25°C had similar results; at the various timepoints, the populations appeared to be nearly at the initial inoculation levels or were below the assay sensitivity limit (Tables 2 and 3). At weeks 6 and 7 of storage at 25°C, the quality of both the fresh apples and the caramel apples was poor, with many of the apples exhibiting surface mold growth.

For *L. monocytogenes* inoculated at 3 log CFU per apple at the stem end and stored at 25°C, approximately 7- to 8-log increases were found on both Gala and Granny Smith caramel apples (Table 3). Because of insufficient data, an accurate growth rate could not be determined for these trials; nevertheless, the increase in population within week 1 of storage was substantial. Surface-inoculated *L. monocytogenes* levels were below the sensitivity of the assay except for one timepoint (Granny Smith, 14 days); however, in all cases the pathogen was detectable by enrichment culture.

Effect of inoculum drying conditions on *L. monocytogenes* populations on caramel apples. In all cases, drying of the inoculum at 5°C for 24 h resulted in lower μ_{max} values and longer times to achieve a 1-log increase in population compared with drying of the inoculum for 2 h at ambient temperature (25°C; compare "stem end, dried" and "stem end," respectively, in Table 2). On Gala apples inoculated with 7 log CFU per apple at the stem end and dried for 2 h at ambient temperature before the addition of a stick and dipping in caramel, *L. monocytogenes* had a μ_{max} value of 0.95 log CFU per apple per day during storage at 5°C; DMFit did not predict a lag phase. However, the apples for which inocula were dried for 24 h at 5°C and then stored at 5°C, DMFit did predict a lag phase (5.8 days) and a significantly lower μ_{max} value (0.80 log CFU per apple per day). Therefore, the *L. monocytogenes* population on the apples in which the inoculum was dried for 2 h at 25°C increased by 1 log CFU in only 26.2 h, compared with 172.8

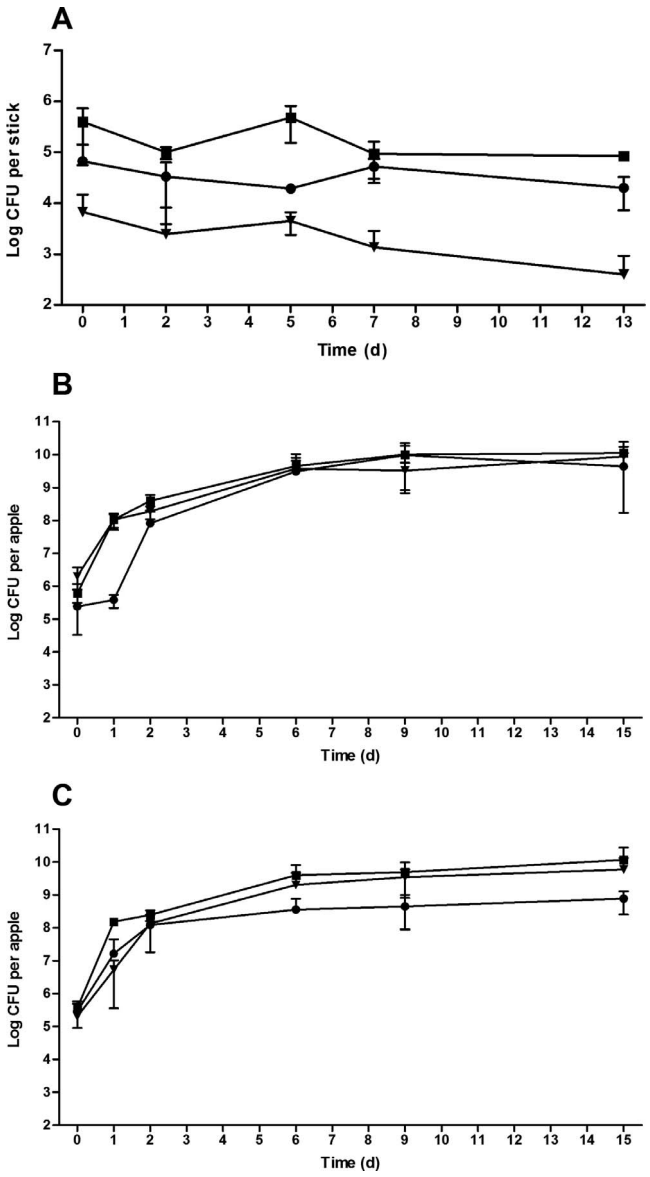

FIGURE 1. *Survival of* L. monocytogenes *on stick materials (A) during storage at 5°C: paper (filled square), wood (filled circle), and plastic (filled triangle). Each data point represents the mean ± SD log CFU per stick (*n = 6*).* L. monocytogenes *survival and growth on Gala (B) and Granny Smith (C) caramel apples with different stick materials during storage at 25°C. Apples were inoculated at the stem end, and a wood stick (closed circle), paper stick (closed square), or plastic stick (closed triangle) was inserted. Each data point represents mean ± SD log CFU per apple (*n = 6*). The sensitivity of the assay was 2.5 log CFU per apple.*

h on the apples that were dried for 24 h at 5°C. Although no other lag-phase values were determined by DMFit for apples inoculated at the stem end and dried for 24 h at 5°C, all the μ_{max} values were significantly lower than those for apples dried for 2 h at 25°C. However, the final population levels at the end of the storage periods were often similar for apples under both inoculum drying scenarios.

***L. monocytogenes* survival on stick materials.** In a comparison of *L. monocytogenes* survival on three caramel apple stick materials (paper, wood, and plastic), survival was significantly better on paper and wood than on plastic (Fig. 1A). After initial inoculation with 7 log CFU per stick, an approximately 1- to 2-log decrease occurred on both paper and wood sticks. A significantly greater decrease, i.e., approximately 3 log CFU, occurred on plastic sticks. After 13 days of incubation at 5°C, overall populations on paper and wood sticks did not decrease significantly, whereas the population on plastic sticks decreased by approximately 1 log CFU. Even though differences in *L. monocytogenes* survival on different stick materials were observed, these differences were diminished when the sticks were used in the preparation of caramel apples (Fig. 1B and 1C). Small but significant differences ($P < 0.05$) in growth of the pathogen on Gala caramel apples stored at 25°C were found when wood, paper, and plastic sticks were used for caramel apple preparation (Table 4). A 3- to 4-log increase in populations of *L. monocytogenes* on Gala and Granny Smith apples was found after 15 days compared with initial levels.

TABLE 4. L. monocytogenes *growth kinetics on caramel apples with wood, paper, or plastic sticks during storage at 25°C for 15 days*[a]

Stick material	Apple variety	$\mu_{max} \pm$ SE[b]	r^2	Time to 1 log CFU growth (h)
Wood	Gala	1.64 ± 0.27 A a	0.89	14.9 ± 0.1
	Granny Smith	1.38 ± 0.20 B a	0.91	17.6 ± 0.2
Paper	Gala	1.40 ± 0.24 A b	0.77	17.4 ± 0.1
	Granny Smith	1.23 ± 0.28 B b	0.71	20.2 ± 0.2
Plastic	Gala	1.02 ± 0.14 A c	0.87	23.9 ± 0.1
	Granny Smith	1.25 ± 0.300 B b	0.73	20.0 ± 0.2

[a] $\mu_{max} \pm$ SE, mean maximum growth rate (log CFU per apple per day) ± standard error; r^2, coefficient of determination.
[b] Means with different uppercase letters are significantly different ($P < 0.05$) for comparisons of different apple varieties with the same stick material. Means with different lowercase letters are significantly different ($P < 0.05$) for comparisons of the same apple variety with different stick materials.

Native microbiota. Populations of certain native microbiota (yeasts, molds, and lactic acid bacteria) that were monitored throughout the storage experiments increased during storage at 25°C for both apple varieties. At 5°C, only the yeast and mold populations increased (Fig. 2). A correlation could not be made between native microflora populations and pH changes in the apples during the storage periods.

DISCUSSION

Although the listeriosis outbreak evaluated here is the first to be attributed to whole caramel apples, studies have shown that *L. monocytogenes* is capable of both surviving and growing on raw fruits such as whole and cut melons *(13, 14, 20, 23, 27),* melon pulp *(26),* cut pears *(1, 11, 23),* and whole and cut berries *(1, 23).* This pathogen also can proliferate on fresh-cut apple slices when contamination occurs after processing procedures, such as peeling and cutting *(2, 4, 12).* For example, the *L. monocytogenes* population on whole Red Delicious apples increased by 0.6 log CFU per apple slice after 7 days of storage at 10°C *(18).*

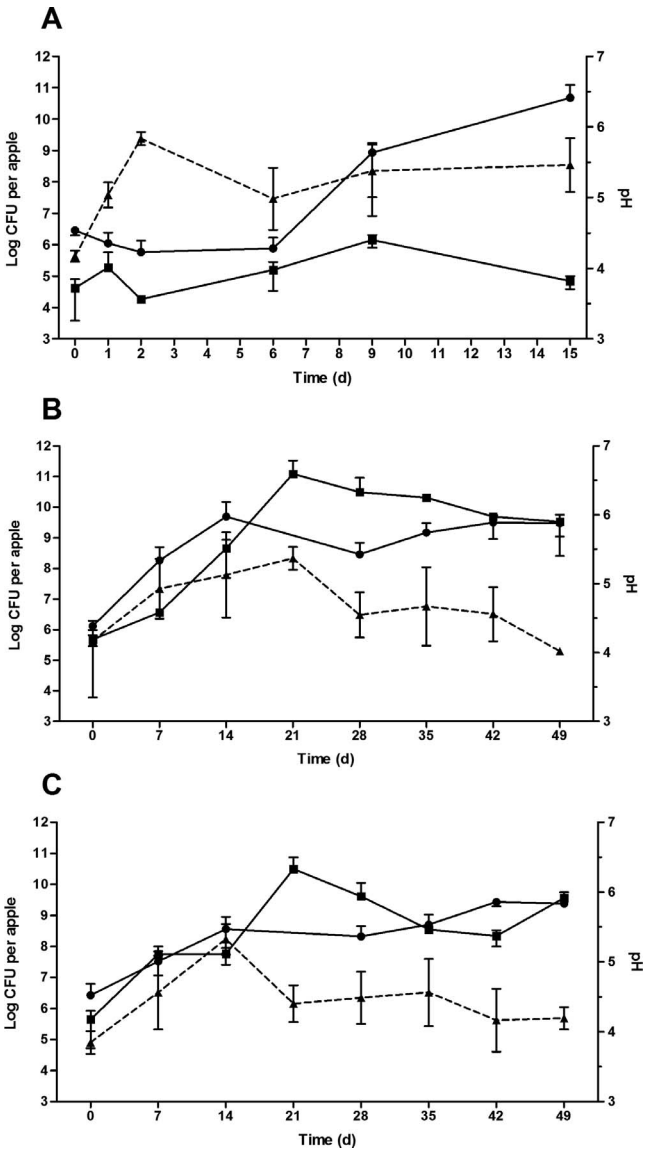

FIGURE 2. *Comparison of pH (closed triangle, dotted line) and native microflora populations (yeasts and molds, closed circle; lactic acid bacteria, closed squares) on (A) control (no inoculation, with caramel coating and stick insertion) Gala apples stored at 5°C for 15 days; (B) control Gala apples stored at 25°C for 49 days, and (C) control Granny Smith apples stored at 25°C for 49 days. Each data point represents mean ± SD log CFU per apple* (n = *6). Sensitivity of the assay was 2.5 log CFU per apple.*

Listeria innocua (a nonpathogenic surrogate for *L. monocytogenes*) increased on Granny Smith and Golden Delicious apple plugs by 2 log CFU per plug after 2 days when stored at either 20 or 25°C; at 10°C, *L. innocua* increased by 2.4 log CFU per plug after 6 days *(2)*. In addition to the increase in *L. monocytogenes* populations, the levels of general microbiota on apples can also increase during storage *(17)*. The results of the present study revealed increases in populations of lactic acid bacteria and yeasts and molds. Yeasts may aid in growth of *L. monocytogenes* and other microorganisms on caramel apples because of their saccharolytic interactions with caramel and apple sugars *(19)*.

In the present study, *L. monocytogenes* inoculation at the stem end of the apple followed by stem end stick insertion and caramel coating including 2 to 3 cm of stick resulted in an environment in which this pathogen was able to both survive and grow. The interface between the stem end of the apple and the caramel layer may produce a microenvironment with high water activity and high nutrient (apple and apple juices produced from the insertion of the stick) and sugar (caramel) concentrations *(16)*. Specific microenvironments of multicomponent foods can affect the survival and thermal behavior of pathogens such as *Salmonella enterica (21)*. In the present study, the apples were submerged in the molten caramel just long enough for the apple and approximately 2 to 3 cm of the stick to be completely covered. *L. monocytogenes* residing in the microenvironment of the stem end of the apple would be exposed to molten caramel at 71 to 88°C in a caramel apple manufacturing plant during the dipping process. The length of time the pathogen is in contact with the caramel could determine, in part, pathogen survival *(15)*. In the present study, some survival curves were highly variable, possibly because of inconsistencies in exposure of the pathogen to the molten caramel. For example, the most inconsistent recovery of *L. monocytogenes* was occurred with surface-inoculated caramel apples. Variable exposure of the pathogen to the thermal effects of the molten caramel could be attributed to location of the inoculum on the apple surface, the temperature of the caramel coating, and the amount of caramel applied, all of which may have produced different microenvironments for the pathogen. The most consistent trends observed in this study occurred with caramel apples inoculated at the stem end. In these apples, some inoculum cells may have been partially protected from thermal exposure by being pushed inside the apple during stick insertion. Nevertheless, the data indicate that manufacturers should not consider hot caramel dip a lethality step sufficient to reduce or eliminate the risk of *L. monocytogenes* contamination on caramel apples.

The mechanism of contamination may be a factor influencing pathogen survival. We studied the effects of two inoculation procedures to mimic two hypothetical contamination scenarios, i.e., contamination prior to cold storage (inoculum drying for 24 h at 5°C) and contamination as a short event at ambient temperature (inoculum drying for 2 h at 25°C). In this study, drying at 5°C slowed the growth of *L. monocytogenes* on caramel apples. In all cases, the inoculum dried for 24 h at 5°C always had a slower growth rate and took longer to achieve a 1-log increase (Table 2). Therefore, for conservative growth models and risk assessments, a 25°C inoculum drying time may be used for data generation. In all of the experiments the *L. monocytogenes* populations may be 2-log higher than indicated because of the efficiency of recovery (see "Materials and Methods"); thus, data depicting the final population levels may be estimated at approximately 2-log higher than the values actually recorded.

During washing of fresh apples, the stem and blossom ends are more difficult to clean than are the smooth surfaces, which is a significant problem *(6, 7, 22)*. Postharvest processing procedures for fresh apples include washes with sanitizers such as chlorine to reduce the total microbial load and to eliminate pathogenic organisms such as *L. monocytogenes*. Once a wound is introduced at the stem end via the

insertion of a stick during caramel apple manufacture, microorganisms may invade the core or flesh and proliferate. This scenario may explain the growth of *L. monocytogenes* during the recent caramel apple outbreak. The Baranyi and Roberts *(3)* model used in this study determined values for growth rate, lag phase, and length of time for a 1-log increase in *L. monocytogenes* on the apples (Table 2). Apples were not washed prior to the experiments to ensure that native microflora remained and would interact with the pathogen in a realistic manner. The data provided a conservative model prediction of time to a 1-log increase in population and risk assessment for *L. monocytogenes* survival and growth on the apples. Apple variety and choice of stick material did not play significant biological roles in the growth of this pathogen on caramel apples. These results provide a starting point for the development of guidelines for caramel apple manufacturers on the safe handling practices of fresh apples and caramel apple products. Many questions remain with respect to potential preventive control options for caramel apple production, which may ultimately depend on the mechanisms by which contamination occurs.

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