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Holding Fresh-Cut Produce under Refrigeration May Not Prevent Pathogen Growth: Implications for Time-Temperature Control to Reduce Risk

ABSTRACT

The U.S. Food and Drug Administration Food Code suggests that holding fresh-cut produce at ≤ 5°C will limit growth of pathogenic microorganisms. Here, we determined whether cucumber, onion, pepper, mango, and tomato supported growth of *Listeria monocytogenes* (LM), Shiga toxin-producing *Escherichia coli* (STEC), and *Salmonella enterica* (SALM) at 5, 10, and 22°C. Produce was surface-pasteurized, diced, inoculated with single-pathogen cocktails, and incubated. Survivors were then enumerated with change in population (Δ-log CFU per gram) determined over time. Mango did not support pathogen growth at 5 or 10°C, but SALM and STEC exhibited significant ($P < 0.05$) growth on mango at 22°C (2.85 and 1.41 Δ-log CFU/g, respectively). At 5°C, significant ($P < 0.05$) growth was seen on cucumber inoculated with SALM and LM; onion and pepper inoculated with LM; and tomato inoculated with STEC. At 10°C, fresh-cut cucumber, onion, and pepper supported significant ($P < 0.05$) increases in SALM, STEC, and LM, along with SALM on tomato; Δ-log ranged from 3.37 (onion, LM) to 5.40 CFU/g (pepper, SALM). Growth of pathogens was not significantly different ($P < 0.05$) at 10 and 22°C for SALM or STEC inoculated onto onion, pepper, cucumber, or tomato. Results suggest that holding fresh-cut produce at or near refrigeration temperatures (5 or 10°C) may not control risk of pathogen growth.

INTRODUCTION

As Americans look to fresh fruits and vegetables as healthy diet choices *(16, 33)*, produce consumption has increased. However, the lack of a lethality step in the manufacture of fresh-cut produce and a global produce distribution network have contributed to increased foodborne illness outbreaks linked to fresh produce *(3)*. *Listeria monocytogenes* (LM), *Salmonella enterica* (SALM), and Shiga toxin-producing *Escherichia coli* (STEC) have been isolated from raw produce *(21, 29, 37–39)* and have been linked to produce-related outbreaks *(1, 8, 24, 35, 46-47)*. In 2015, *Salmonella* Poona was identified in an outbreak associated with cucumber that resulted in >900 cases of illness, 204 hospitalizations, and six deaths *(4, 5)*. In 2011, an outbreak of listeriosis traced to

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whole cantaloupe caused 146 illnesses in 28 U.S. states and led to 32 deaths and one miscarriage *(30)*.

The U.S. Food and Drug Administration (FDA) Food Code aims to ensure safe food handling and preparation in retail food establishments across the United States. The Food Code identifies certain foods as time-temperature control for safety (TCS) foods; these foods require time-temperature control to limit growth or toxin formation by pathogenic microorganisms *(43)*. TCS foods specifically cited in the definition are raw seed sprouts, cut melons, cut leafy greens, and cut tomatoes or mixtures of cut tomatoes that are not modified in a way so that they are unable to support pathogenic microorganism growth or toxin formation *(43)*. Foods not specifically cited are designated non-TCS on the basis of pH and water activity *(Table 1)*. Except during preparation or similar steps, or under certain holding conditions, TCS foods must be maintained at ≤5°C *(43)*. Most fruit have a natural or normal pH < 4.2 and would be considered non-TCS. Exceptions are cut melon and cut tomato that are specifically cited in the definition of TCS foods. Vegetables tend to have pH > 4.2 and a_w > 0.92 and would require a product assessment to determine the need for TCS *(Table 1)*.

The goal of this project was to determine the impact of holding temperature on the survival of SALM, STEC, and LM on fresh-cut cucumber, onion, pepper, and mango. Produce items were held at or near refrigeration temperature (5 or 10°C) or at room temperature (22°C). Cut tomato, listed as a TCS food in the Food Code, were included as a positive control. We aimed to determine whether holding under refrigeration would limit pathogen growth on a variety of produce as expected and to establish the risk that may be posed to public health by the presence of pathogens on fresh-cut produce held under typical retail handling conditions.

MATERIALS AND METHODS

Produce preparation

Whole produce, procured from local grocery stores (Madison, WI) included tomato, cucumber, onion, green pepper, and mango. Produce items were held at 4 ± 2°C before use in experiments. Whole items were surface pasteurized by dipping in boiling water (100°C) for 30 s and then aseptically peeled (tomato, cucumber, onion, and mango) and chopped into 1-cm cubes by using an alcohol-sanitized vegetable chopper. Chopped flesh from each produce type was collected in a sterile beaker, mixed, and dispensed into sterile Whirl-Pak filter bags (15.2 by 22.9 cm; Nasco, Ft. Atkinson, WI) at 25 g per bag. The pH of each produce item was measured at the beginning of each trial by using a calibrated meter (Accumet AB150, Fisher Scientific, Itasca, IL). Water activity was measured at the initiation of the study, once per produce item, by using a calibrated meter (Aqualab, Meter Group, Pullman, WA).

Pathogen selection and maintenance

Pathogen strains originally isolated from produce or produce-related outbreaks were used in this study *(Table 2)*. Strain identity was confirmed by evaluating Gram reaction, cell morphology, and biochemical profile (API20E and API Listeria, bioMérieux, Durham, NC). Stock cultures for each strain were maintained frozen (-20°C) in tryptic soy broth (TSB; Difco, BD, Sparks, MD) containing 50% glycerol (v/v) (Fisher Scientific).

Working cultures were prepared by streaking for isolation from frozen stock cultures onto selective media. STEC and SALM strains were streaked onto Levine's eosin methylene blue agar (Difco, BD) modified with the addition of 10 g/L D-sorbitol (Fisher Scientific) and 5 g/L NaCl (MEMB; Fisher Scientific); LM strains were streaked onto *Listeria* selective agar (LSA; Oxoid Ltd., Basingstoke, England) with added *Listeria* selective supplement (Oxoid Ltd.). Working culture plates were incubated at 35°C for 24 ± 2 h and then stored at 4°C and refreshed monthly.

Inoculum preparation

Each single-pathogen inoculum cocktail was prepared by picking a single isolated colony from a working culture plate for each strain into a separate tube containing 9 mL of TSB

TABLE 1. Interaction of pH and water activity for control of vegetative cells and spores in food not heat treated or heat treated, but not packaged *(43)*

a_w value	pH < 4.2	pH 4.2–4.6	pH > 4.6–5.0	pH > 5.0
< 0.88	Non-TCS food[a]	Non-TCS food	Non-TCS food	Non-TCS food
0.88–0.90	Non-TCS food	Non-TCS food	Non-TCS food	PA[b]
0.90–0.92	Non-TCS food	Non-TCS food	PA	PA
0.92	Non-TCS food	PA	PA	PA

[a]TCS food means time-temperature control for safety of food.
[b]PA, product assessment required.

TABLE 2. Pathogen strains used in this study

Species	Serotype	Strain or designation	Origin	Source[a]
Escherichia coli	O111:H-	RC-313-007		WSLH
	O145	99-33-11		WSLH
	O103:H2	RC-289-003		WSLH
	O104:H4	RC-401-014	Sprouts-linked outbreak, Germany	WSLH
	O26:H11	RC-431-068	Sprouts-linked outbreak	WSLH
	O157:H7	E0018	Calf feces	UGA
	O157:H7	C7929	Apple cider	UGA
	O157:H7	F4546	Sprouts	UGA
	O157:H7	H1730	Lettuce	UGA
	O157:H7	SEA 13B88	Apple cider	UGA
Salmonella enterica	Agona		Alfalfa sprouts	UGA
	Baildon		Patient isolate (tomato-linked outbreak)	UGA
	Gaminara		Orange juice	UGA
	Michigan		Cantaloupe	UGA
	Montevideo		Patient isolate (tomato-linked outbreak)	UGA
		17 7114A	Green peas (outbreak strain)	WSLH
		17 7114B	Green peas (outbreak strain)	WSLH
		752072	Green peas (outbreak strain)	WSLH
		722078	Green peas (outbreak strain)	WSLH
Listeria monocytogenes	1/2a	H0222	Potato	UGA
	4b	G1091	Patient isolate (coleslaw-linked outbreak)	UGA
	1/2a	F8369	Corn	UGA
	1/2b	F8255	Peach and plum	UGA
	4b	F8027	Celery	UGA

[a]WSLH, Wisconsin State Laboratory of Hygiene; UGA, University of Georgia, courtesy of L. Beuchat.

plus 1% glucose. Cultures were incubated for 24 ± 2 h at 35°C. After incubation, 1 mL from each strain was combined in a single 15-mL conical centrifuge tube to create three single-pathogen cocktails.

Tubes containing multiple strains were centrifuged (Marathon 21K, Fisher Scientific) at 8,000 rpm for 7 min. The supernatant from each tube was discarded, and each pellet was resuspended in Butterfield's phosphate diluent (BPD; 3M, Minneapolis, MN) to create a single-pathogen inoculum cocktail of ~10^9 CFU/mL. Cocktails were serially diluted in BPD, as appropriate, to achieve an inoculum concentration of ~10^4 CFU pathogen per gram of fresh produce.

Produce inoculation and incubation

For a given trial, bags of cut retail produce (25 g), prepared as described above, were individually inoculated with 250 µL of a single-pathogen cocktail of SALM, STEC, or LM to yield an average starting inoculum concentration of 4.78, 4.77, or 4.15 CFU/g, respectively, across three complete trials. Three bags remained uninoculated and were used for enumeration of native microflora at time zero. Once inoculated, bags were gently massaged by hand for 30 s to disperse the inoculum and then placed into an incubator set at 5 ± 1, 10 ± 1, or 22 ± 1°C.

Bacterial enumeration

Surviving pathogens were enumerated from one bag for each pathogen-produce combination (three bags per produce type) at select time-temperature combinations as follows: at 5°C, enumeration at 0, 24, 48, 72, 96, 120, and 168 h; at 10°C, enumeration at 0, 12, 24, 48, 72, and 96 h; and at 22°C, enumeration at 0, 4, 8, 18, 24, and 32 h. For an inoculated bag, at each sampling time, 99 mL of BPD was added, and the

bags were stomached at 260 rpm for 2 min. An aliquot of the resulting homogenate was serially diluted in BPD and spread plated for enumeration. Total microbiota were enumerated on nonselective media: tryptic soy agar (0.1 mL aliquots; Difco, BD) and TPC Petrifilm (1 mL; 3M). Pathogens were enumerated on selective media (0.1 mL): MEMB for SALM and STEC and LSA for LM. Plates were incubated for 24 to 48 h at 35 ± 1°C, and typical colonies were then counted. Surviving native microbiota levels were determined by subtraction. Three independent trials were conducted for each produce-pathogen-temperature combination.

Statistical analysis

Pathogen levels (log CFU per gram) were plotted over time for each produce item at a given temperature. The Combase DMFit model using the integrated model of Baranyi and Roberts (https://www.combase.cc/index.php/en/) was used to generate rates of growth (μ; log CFU per gram per hour). Δ-log CFU per gram was calculated by subtraction: $time_{final} - time_0$. Bacterial level (log CFU per gram) at each sampling time for replicate trials ($n = 3$) was compared using the Sen-Adichie test for parallelism calculated in Mstat version 6.4.2 (https://mcardle.wisc.edu/mstat/). Growth rates and Δ-log CFU per gram values for a given produce-pathogen combination across temperatures and Δ-log CFU per gram values across pathogens for a given produce-temperature combination were compared using one-way analysis of variance (ANOVA) followed by unpaired *t*-test by using Prism version 7.00 for Windows (GraphPad Software, La Jolla, CA; www.graphpad.com). Differences are considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Produce items chosen for this study were selected based on their association with foodborne illness outbreaks or ready availability in fresh-cut form in retail food establishments. Multiple illness outbreaks in the United States and elsewhere have been linked to onion *(48)*, pepper *(25, 32)*, mango *(9, 23, 40)*, cucumber *(4, 5, 14)*, and tomato *(10, 11)*.

Excluding mango, which did not support pathogen growth overall, the rate of growth for pathogen-produce combinations across temperatures followed the trend 5°C < 10°C < 22°C *(Table 3)*, and, except for tomato inoculated with LM, the rate of growth at 5°C < rate of growth at 22°C ($P < 0.05$; *Table 3*). Similarly, Fang et al. *(20)* modeled the growth of different serotypes of LM and background microbiota on fresh-cut cantaloupe and found that temperature was the only factor that significantly affected specific growth rate.

Product pH strongly influences the survival and growth of pathogens on fresh and fresh-cut produce. The average initial pH of retail produce items ranged from 3.58 for mango to 5.67 for cucumbers, and water activity ranged from 0.993 to 0.998 *(Table 4)*. The combination of a short thermal pasteurization and peeling, where used, reduced native microbiota to 1 to 3 log CFU/g *(Table 4)* with no discernible affect on product quality (data not shown).

The FDA Food Code outlines characteristics of pH and water activity that define whether a product requires TCS *(Table 1)* and directs that TCS foods be held at ≤5°C to "… limit pathogenic microorganism growth or toxin formation" *(43)*. Of the produce items included in this study, only mango (pH 3.58) would be considered non-TCS, i.e., not requiring time and temperature control.

Mango did not support growth of any pathogens on holding at 5°C, as expected, nor at 10°C *(Table 3)*. However, placing fresh-cut mango at 22°C and holding for up to 32 h allowed significant growth of SALM and STEC (Δ-log CFU/g of 2.85 and 1.41, respectively). LM inoculated on fresh-cut mango did not grow over 32 h at 22°C *(Table 3)*. Strawn and Danyluk *(41)* investigated the fate of *Escherichia coli* O157:H7 and *Salmonella* spp. on fresh-cut mango inoculated and held at 4, 12, and 23°C. Unlike our results, they observed growth of SALM inoculated on mango at 12°C, and pathogen populations increased over the first day on incubation at 4°C. Similar to our study, however, E. coli O157:H7 inoculated onto mango slices held at 4 or 12°C were virtually unchanged *(41)*. At 23°C, they observed an increase in SALM populations on mango slices of up to 3 log CFU/g within the first 24 h, similar to the 2.85 log CFU/g we observed in SALM over 32 h at 22°C. We observed a significant ($P < 0.05$) increase in STEC on fresh-cut mango at 22°C (Δ-log 1.41 log CFU/g), whereas Strawn and Danyluk *(41)* did not observe sustained growth of this pathogen on fresh-cut mango held at 23°C. Differences in strain selection and preparation or study design, and slight differences in incubation temperature, may account for variation in pathogen response between the two studies. Importantly, data presented by the two studies indicate the potential for STEC and SALM strains to survive and grow on cut mango under refrigeration (≤5°C) and at room temperature (22 to 23°C), suggesting that if mango became contaminated during processing and was subsequently held, public health may be placed at risk. In addition, holding fresh-cut mango at 4 or 5°C, although limiting growth of some pathogen strains, would not ensure safety because a documented log reduction in pathogens over time was not observed in either study.

Although holding fresh-cut onion at 5°C prevented statistically significant growth of SALM and STEC, more than 1-log growth of LM was observed (*Table 3*, 1.37 log CFU/g growth over 168 h). This confirms the previous work of Neetoo et al. *(34)* wherein *Salmonella* and *E. coli* O157:H7 were unable to grow on green onion at 4°C, but inocula survived for up to 14 days with no significant change in population. Placing fresh-cut onion at an abusive refrigeration temperature (10°C) resulted in significant ($P < 0.05$) growth of SALM, STEC, and LM, with increases

TABLE 3. Average pathogen growth rate and change in log of *Salmonella* (SALM), Shiga toxin-producing *E. coli* (STEC), and *L. monocytogenes* (LM) on fresh-cut onion, pepper, mango, cucumber, and tomato held at 5, 10, and 22°C (*n* = 3)[a]

Storage temp for produce item	SALM		STEC		LM	
	μ[b]	Δ-log[c]	μ	Δ-log	μ	Δ-log
5°C						
Onion	0.00 a	0.25 aA	0.00 a	−0.19 aA	0.02 a	1.37 aB
Pepper	0.00 a	0.23 aA	0.00 a	0.02 aA	0.02 a	2.36 aB
Mango	−0.01 a	−0.50 aA	0.00 a	−0.01 aA	0.00 a	−0.05 aA
Cucumber	0.04 a	1.30 aA	0.02 a	0.39 aB	0.03 a	1.96 aA
Tomato	0.00 a	0.27aA	0.02 a	0.71aA	0.00a	−0.18 aB
10°C						
Onion	0.11 b	5.10 bA	0.08 b	4.94 bA	0.04 b	3.37 bB
Pepper	0.09 b	5.40 bA	0.09 b	5.07 bA	0.08 b	4.83 bA
Mango	−0.01 a	0.00 aA	0.00 a	0.07 aA	0.00 a	0.01 aA
Cucumber	0.08 a	5.24 bA	0.10 ab	5.25 bA	0.06 ab	4.19 bB
Tomato	0.08 b	4.36 bA	0.06 ab	2.87 abA	0.00 a	0.04 aB
22°C						
Onion	1.10 c	4.63 bAB	0.88 c	4.98 bA	0.52 c	4.04 cB
Pepper	0.80 c	5.66 bA	1.01 c	5.42 bA	0.55 c	4.77 bA
Mango	0.15 a	2.85 bA	0.08 a	1.41 bAB	−0.05 a	−0.44 aB
Cucumber	0.45 b	5.28 bA	0.43 b	5.27 bA	0.31 b	4.64 bA
Tomato	0.48 c	5.17 bA	0.31 b	4.45 bA	0.06 a	0.54 aB

[a]Values for pathogen growth rate (μ) are given in log CFU/g/h and change in log (Δ-log) as CFU/g. Different lowercase letters appearing in a column indicate significant differences among growth rate or Δ-log for a given pathogen-produce combination across different temperatures ($P < 0.05$). Different uppercase letters appearing in a row indicate significant differences among Δ-log across pathogens for a given produce-temperature combination ($P < 0.05$).

[b]μ, growth rate (log CFU/g/h).

[c]Δ-log, change in log CFU/g $time_x - time_0$; x = 168 h at 5°C, 96 h at 10°C, and 32 h at 22°C.

TABLE 4. Intrinsic characteristics of produce items used in this study

Produce item	Avg pH[a]	a_w	Native microbiota (log CFU/g)
Cucumber	5.67 (5.46–5.88)[b]	0.998	2.93
Mango	3.58 (3.23–3.88)	0.993	1.41
Onion	5.47 (5.39–5.64)	0.994	1.21
Pepper	5.60 (5.42–5.77)	0.994	2.24
Tomato	4.44 (4.32–4.56)	0.993	1.24

[a]Number of independent replicates (*n* = x): pH *(3)*, a_w *(1)*, average level of native microbiota at the start of each storage study *(3)*.

[b]Range across three independent trials.

of 5.10, 4.94, and 3.37 log CFU/g over 96 h, respectively. Elevating temperature to 22°C and holding for 32 h did not lead to a significant ($P > 0.05$) increase in SALM or STEC populations compared with 10°C but did allow for significant ($P < 0.05$) growth of LM *(Table 3)*. Similarly, Neetoo et al. *(34)* observed population increases for *E. coli* O157:H7 and *Salmonella* of 3.8 to 4.7 and 3.6 to 4.6 log CFU/g, respectively, over an 8-day incubation period at 22°C. Others have observed growth of LM on diced onion under fluctuating temperature scenarios *(27)*.

Pathogen response to temperature when inoculated onto fresh-cut pepper was similar to that of onion, with significant growth of LM at 5°C (2.36 log CFU/g growth over 168 h), but not of SALM and STEC, and a dramatic increase in population across all pathogens at 10°C (Δ-log CFU/g of 5.40, 5.07, and 4.83 over 96 h for SALM, STEC, and LM, respectively) *(Table 3)*. Growth of pathogens on fresh-cut pepper at 22° was not significantly ($P > 0.05$) different from 10°C. Castro-Rosas et al. *(17)* similarly noted growth of *Salmonella* and *E. coli* on sliced chili pepper at 25°C, with growth of ~4 log CFU on pepper slices after 24 h. Unlike our results, however, Castro-Rosas et al. *(17)* observed a 1- to 2-log CFU reduction in population of *Salmonella* or *E. coli* strains on pepper slices after 6 days at 3°C, presumably due to differences in either bacterial strains or incubation temperature, or perhaps due to antibacterial activity associated with sliced chilies *(28)*. Castro-Rosas et al. *(17)* noted that survival of even a small number of *Salmonella* or other pathogens under refrigeration presents a serious health hazard to consumers due to the low infectious dose associated with many foodborne pathogens.

Holding of fresh-cut cucumber at 5°C allowed for significant growth of both SALM and LM (Δ-log CFU/g of 1.30 and 1.96, respectively) *(Table 3)*. As seen with onion and pepper, increasing hold temperature to 10°C significantly ($P < 0.05$) increased pathogen growth over 5°C. Holding inoculated fresh-cut cucumbers for 96 h at 10°C resulted in values of 5.24, 5.25, and 4.19 Δ-log CFU/g for SALM, STEC, and LM, respectively. Whereas growth of SALM and STEC at 22°C was not significantly ($P > 0.05$) different from 10°C, growth of LM was significantly higher at 22°C on fresh-cut cucumber (4.64 log CFU/g) over 32 h *(Table 3)*. Bardsley et al. *(7)* found that LM populations significantly increased on sliced cucumbers (2.9 log CFU/g) held at 4°C, whereas *Salmonella* populations significantly decreased (1.3 log CFU/g). The extended hold time in the Bardsley et al. *(7)* study (21 days) may account for the difference in results. Our results are, however, consistent with what others have observed for survival patterns of SALM and LM inoculated into cucumber tissue stored at 10°C *(18)*, 23°C *(7)*, or 25°C *(22)*, but contrary to what has been observed for *Salmonella*-inoculated cucumber slices stored 48 h at 4°C, wherein no change in pathogen population was observed *(22)*. The difference in study results can be explained by a difference in strain selection and study methodology. As with other produce items in this study, pathogen population numbers on chopped cucumber did not decrease on holding at 5°C; instead; at least a doubling of inoculum population density was observed for SALM, STEC, and LM (Δ-log > 0.3 log CFU/g) *(Table 3)*. The ability of LM and SALM to grow on sliced cucumbers in short amounts of time at ambient temperatures (1 day) and to survive on sliced cucumbers past the recommended shelf life at refrigeration temperatures highlights the need to reduce the likelihood of contamination events throughout the cucumber supply chain *(7)*.

Tomato is listed as a TCS food in the FDA Food Code and was included in this study as a positive control. Tomato tissue pH averaged 4.4 across trials. As expected, holding fresh-cut tomatoes for up to 196 h at 5°C did not allow for significant pathogen growth *(Table 3)*. At an abusive refrigeration temperature (10°C), there was significant ($P < 0.05$) growth of SALM on fresh-cut tomato (4.36 log CFU/g over 96 h) and a detectable increase in STEC that proved not significant ($P > 0.05$). At 22°C, both SALM and STEC grew robustly on fresh-cut tomato (Δ-log CFU/g of 5.17 and 4.45, respectively) over 32 h.

Beuchat et al. *(12, 13)* have investigated pathogen growth in tomato flesh. Acid-adapted and nonadapted strains of *Salmonella* were inoculated into 'Roma' tomato pulp (pH ~ 4.3) that was subsequently stored at 4, 12, and 21°C. *Salmonella* populations increased ~2 log CFU/g over 10 days at 12°C, but no population increase was observed at the first sampling point (3 days). A population increase of 3 to 4 log CFU/g was noted by day 3 for tomato pulp stored at 21°C, with little further change noted from day 3 to day 10. We observed greater increases in SALM populations inoculated onto fresh-cut tomatoes and held at 10 or 22°C, likely due to differences in study methodology. Separate investigations into survival of *E. coli* O157:H7 inoculated into processed whole tomato (canned, peeled whole tomatoes, no other ingredients, pH 4.6) did not show marked variation in pathogen count at 4°C over time. At 25°C, however, the population of test strains increased by ~3 log CFU/g in the first 48 h *(19)*, survival trends similar to what we observed.

Fresh-cut tomato did not support significant growth of LM at any holding temperature *(Table 3)*. When Beuchat et al. *(12)* investigated survival of LM on chopped tomatoes held at 10 or 21°C, they observed no population changes over the first 4 days of storage at 10°C and an ~1-log population decrease at 21°C over the first 48 h. In the same study *(12)*, when LM was inoculated into commercially processed tomato juice (pH 4.21), pathogen population did not change when held at 5°C for 7 days.

Holding fresh-cut produce for 196 h at 5°C failed to prevent significant ($P < 0.05$) growth of LM on onion, pepper, and cucumber (Δ-log CFU/g of 1.37, 2.36, and 1.96, respectively). Significant growth of SALM on cucumber was also observed (*Table 3*, 1.30 log CFU/g). Overall, growth of pathogens on fresh-cut produce was not significantly different ($P < 0.05$) at 10 and 22°C for SALM and STEC

inoculated onto onion, pepper, cucumber, and tomato *(Table 3)*. Typical patterns observed for pathogens survival in fresh-cut produce held at 5°C are shown for SALM and LM in *Fig. 1a and 1b*, respectively. Typical growth patterns of SALM at 10°C on fresh-cut produce are shown in *Fig. 2*.

Researchers *(26, 50)* have noted that incubation temperature and nutrient availability are major factors affecting pathogen growth and thus food safety risks associated with fresh-cut produce. The concept of time-temperature control for fresh produce, particularly fresh-cut fruits and vegetables, has historically been relied upon to maintain quality and extend the shelf life of whole produce and fresh-cut products *(31, 44)*. The application of TCS as part of the FDA Food Code *(43)* is intended to prevent the growth of, or toxin formation by, pathogenic microorganisms. If time without temperature control is

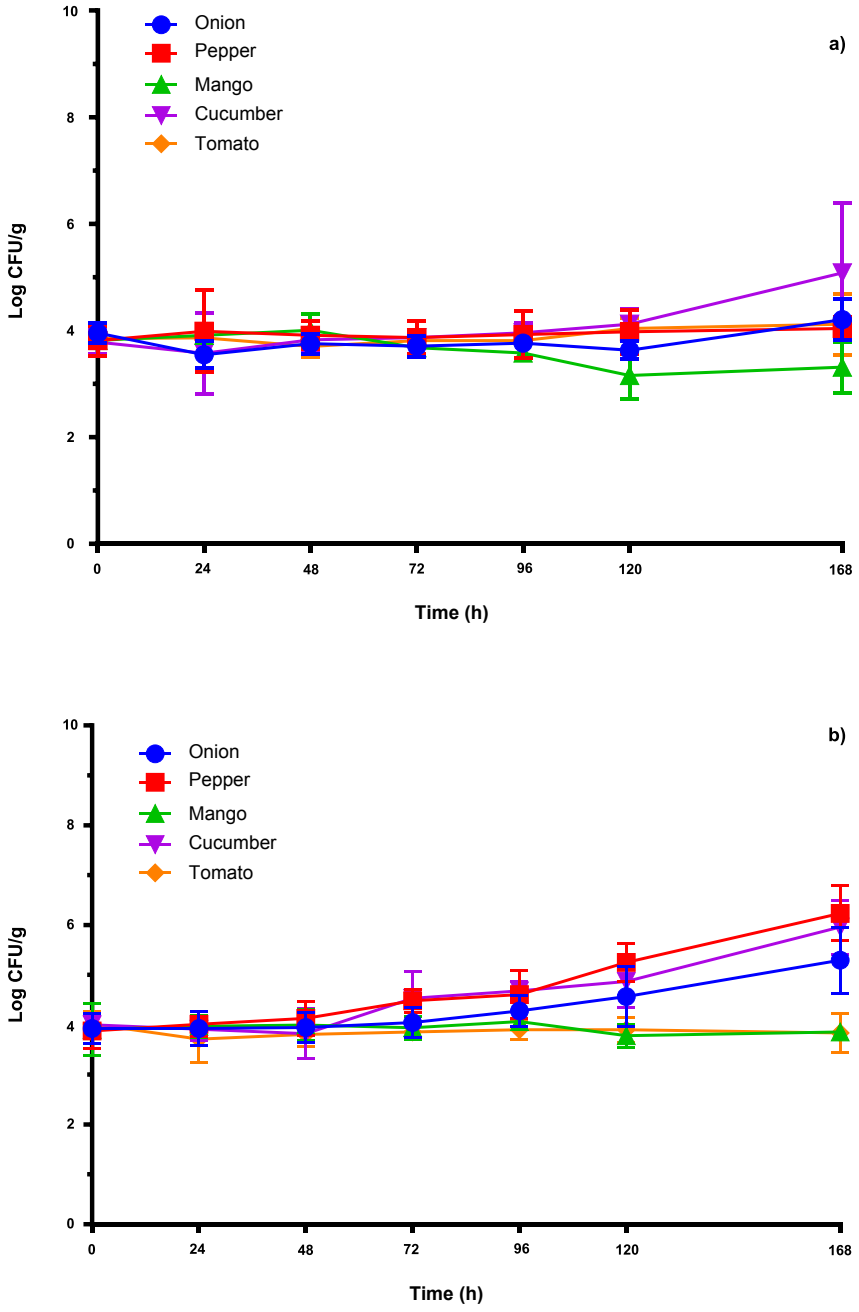

FIGURE 1. Behavior of pathogens (a) *Salmonella* spp. and (b) *L. monocytogenes* inoculated on fresh-cut produce items stored at 5°C for up to 168 h. Bacterial populations (log CFU per gram) are the means of replicate trials (n = 3); error bars represent standard deviations.

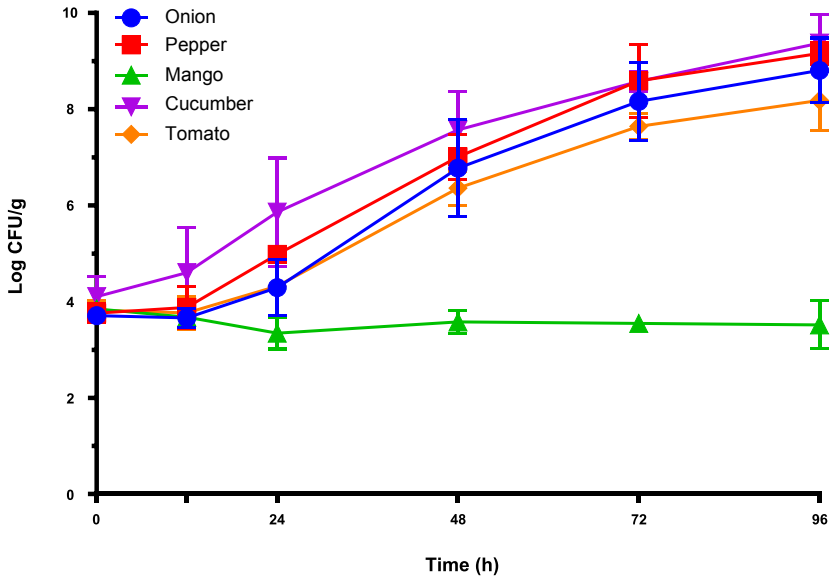

FIGURE 2. Behavior of *Salmonella* on fresh-cut produce items stored at 10°C for up to 96 h. Bacterial populations (log CFU/g) are the means of replicate trials (n = 3); error bars represent standard deviations.

used as the public health control, food that is displayed or held for sale or service may be held for a period of 4 to 6 h, depending on ambient temperature, after which point the product must be cooked and served, consumed, or discarded. When food is prepared on-site at a retail establishment and held for more than 24 h on-site, the item must be clearly date marked and held at ≤5°C for no more than 7 days *(43)*. A food item that is prepared and packaged by a food processing plant, and not a retail food establishment, may be held at the retail establishment, once opened, for a time not to exceed the manufacturer's use-by date based on food safety; holding at ≤5°C is required if the item is TCS. Our results suggest that on holding at >5°C, fresh-cut onion, pepper, mango, and cucumber all support growth of at least one pathogen that is reasonably likely to occur on the raw product. In addition to tomato that is already designated a TCS food, each of these fresh-cut items would reasonably be designated as a TCS food.

As McEntire *(31)* noted, good manufacturing practices under the Food Safety Modernization Act state, in part, in 21 CFR 117:

(b) *Manufacturing operations.* (2) All food manufacturing, including packaging and storage, shall be conducted under such conditions and controls as are necessary to minimize the potential for the growth of microorganisms, or for the contamination of food. One way to comply with this requirement is careful monitoring of physical factors such as time, temperature, humidity, a_w, pH, pressure, flow rate, and manufacturing operations such as freezing, dehydration, heat processing, acidification, and refrigeration to ensure that mechanical breakdowns, time delays, temperature fluctuations, and other factors do not contribute to the decomposition or contamination of food.

(3) Food that can support the rapid growth of undesirable microorganisms, particularly those of public health significance, shall be held in a manner that prevents the food from becoming adulterated within the meaning of the act. Compliance with this requirement may be accomplished by any effective means, including: (i) Maintaining refrigerated foods at 45°F (7.2°C) or below as appropriate for the particular food involved *(31, 45)*.

McEntire *(31)* notes that essentially good manufacturing practices require the food industry to take measures to minimize the growth of pathogens in food. However, the food industry lacks information on which specific fresh fruits and vegetables support pathogen growth, thereby complicating the application of risk-based preventive controls such as time and temperature. And, given the broad number of intrinsic and extrinsic factors that affect pathogen growth on fresh-cut produce *(26, 36)*, we would argue that application of appropriate food safety controls for fresh-cut produce can be complicated, especially at the consumer-retail interface. In some instances, the fresh-cut produce items that we studied supported pathogen growth even held under refrigeration (5°C). Evaluating risk at the manufacturing level or at retail should include the consideration that fresh-cut produce may support the growth of pathogens, even when proper temperature controls are in place. Manufacturers and retailers intent on minimizing risk may need to consider additional interventions, i.e., heat or antimicrobial treatment, to protect public health.

Also notable in this study is that although pathogen growth was often not observed at 5°C, temperature control did not result in any dramatic decrease in pathogen populations. The Δ-log values at the end of incubation at 5°C (7 days) suggest

that pathogens that find their way onto fresh-cut produce may survive during retail display and, in the likely absence of an intervention treatment applied by the consumer, could present a threat to public health. In the case of LM inoculated onto fresh-cut onion or pepper, or LM or SALM onto fresh-cut cucumber, there is the distinct possibility of an increased, not decreased, risk on holding at 5°C for up to the allowed 7 days. Were holding temperature to increase to 10°C, at least episodically, or even the 7.2°C allowed under the Preventive Controls for Human Foods Rule *(45)*, a situation not unlikely in retail display *(29, 36)*, there is a heightened risk of pathogen presence on fresh-cut produce purchased by consumers. *E. coli* O157:H7 has been implicated in recent outbreaks of infections associated with fresh fruits and vegetables, and evidence suggests that holding temperature regimes normally thought to protect public health (< 7°C) are commonly exceeded during transport or retail display *(2, 27, 29, 37)*.

Also noted in this study was the growth of SALM and STEC on mango on holding at 22°C. Cut mango has a pH < 4.2 and, as a non-TCS food, regulatory decision-making would support holding at >5°C for extended periods, presumably without increasing risk. Our results, however, suggest that fresh-cut produce, including high-acid fruits, if contaminated and held at >5°C could, in some instances, present a risk to public health.

Given the association between fruit and vegetable consumption and positive health outcomes *(33, 42)*, the Dietary Guidelines for Americans encourage increased fruit and vegetable consumption across all age groups *(42)*. At the same time, older adults, the very young, and the immune compromised are more likely to have some of the most severe complications from foodborne pathogen infections *(15)*; therefore, these populations face heightened risk from consumption of contaminated fresh and fresh-cut produce. A recent report *(6)* of an investigation into a salmonellosis outbreak traced to fresh-cut fruits that sickened more than 150 people in 14 states noted that one of the factors likely contributing to the high hospitalization rate (66% of confirmed cases) was the types of facilities that received the fruit, i.e., long-term care facilities, hospitals, and schools. For individuals in these vulnerable populations, any increase in pathogen population, or even the ability for existing pathogens to survive during retail holding or display, could present an unacceptable health risk. Certainly, our data support observations made by others that strategies aimed at reducing pathogen prevalence and concentration on produce growing in the field or orchard, or during processing and handling, are important in risk control *(7, 37, 41)*.

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