

Growth of *Salmonella enterica* and *Listeria monocytogenes* on Fresh-Cut Cantaloupe under Different Temperature Abuse Scenarios

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ABSTRACT

Effective cold chain management is a critical component of food safety practice. In this study, we examined the impact of commonly encountered temperature abuse scenarios on the proliferation of *Salmonella enterica* and *Listeria monocytogenes* on fresh-cut cantaloupe. Inoculated fresh-cut cantaloupe cubes were subjected to various temperature abuse conditions, and the growth of *S. enterica* and *L. monocytogenes* was determined. During 1 week of storage, *Salmonella* cell counts on fresh-cut cantaloupe increased by -0.26 , 1.39 , and 2.23 log units at 4°C (control), 8°C , and 12°C (chronic temperature abuse), respectively, whereas that of *L. monocytogenes* increased by 0.75 , 2.86 , and 4.17 log units. Under intermittent temperature abuse conditions, where storage temperature fluctuated twice daily to room temperature for 30 min, *Salmonella* cell count increased by 2.18 log units, whereas that of *L. monocytogenes* increased by 1.86 log units. In contrast, terminal acute temperature abuses for 2 to 4 h resulted in upwards to 0.6 log unit for *Salmonella*, whereas the effect on *L. monocytogenes* was less significant compared with *L. monocytogenes* on cut cantaloupe stored at 4°C . Significant deterioration of produce visual quality and tissue integrity, as reflected by electrolyte leakage, was also observed under various temperature abuse conditions.

In recent years, netted melons such as cantaloupes have emerged as one of the most significant vehicles of widespread foodborne illness outbreaks caused by bacterial pathogens (17). In 2011, contaminated cantaloupes from one farm in Colorado caused a multistate outbreak of *Listeria monocytogenes* infections that sickened at least 147 people in 28 states, including 143 hospitalizations and 33 deaths (3, 18). In 2012, a large-scale outbreak of salmonellosis caused by *Salmonella enterica* serovars Typhimurium and Newport resulted in 261 reported infections in 24 states, including 94 hospitalizations and 3 deaths reported to the Centers for Disease Control and Prevention (4). Since 2000, at least 29 multistate outbreaks of foodborne infections by *S. enterica* serovars, enterohemorrhagic *Escherichia coli*, or *L. monocytogenes* associated with melons have been reported in United States and Canada. At least 18 of these outbreaks were *Salmonella* outbreaks that implicated cantaloupes (Division of Public Health Risks Science, Laboratory of Foodborne Zoonoses, Public Health Agency of Canada, unpublished data).

Cantaloupes are grown in direct contact with soil, potentially resulting in higher vulnerability to contamination by microorganisms, including bacterial pathogens, from soil, soil amendments, irrigation water, and intruding

wildlife (6, 7, 14). Microstructures of the netting give the cantaloupe rind inherent surface roughness that is likely to favor bacterial attachment (25). The topographic characteristics of cantaloupes and other netted melons can provide extra protection to microorganisms (30). Unlike the smooth-skinned melons, such as honeydews and watermelons, the extensive netting on the surface of cantaloupes increases the attachment by bacteria and reduces the efficacy of commonly used postharvest interventions, such as washing with chlorinated water (23, 29). Indeed, biofilm formation has been reported for bacterial pathogens on cantaloupe surface nettings and is considered the main reason of ineffectiveness of chemical sanitizers for reducing *Salmonella* contamination on the cantaloupe surface (2, 24).

In commercial operations, cantaloupes are subjected to various antimicrobial treatments, such as chlorinated water or brief surface pasteurization, before the removal of rinds and packaging of fresh-cut flesh (1). The relatively low efficacy of postharvest antimicrobial interventions for cantaloupes determines the critical importance for effective cold chain management of fresh-cut cantaloupes. Effective cold chain management is also critical for maintaining the quality and shelf life of the products.

Cold chain management is a critical component of quality and safety control for all fresh-cut produce (8). For leafy greens, a broken cold chain can result in rapid growth of spoilage and, if present, foodborne pathogens (15). The

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growth of inoculated bacterial pathogens such as *E. coli* O157:H7 can outpace that of the spoilage bacteria and the appearance of quality deterioration, resulting in unsafe product without visual aversion (16). Fresh-cut cantaloupe is rich in sugars and other nutrients on the cut surfaces that necessitate stringent temperature control to prevent potential proliferation by bacterial contaminants. In this study, we investigated the survival and growth of *S. enterica* serovars and *L. monocytogenes*, two bacteria that were found in recent cantaloupe-associated outbreaks, on fresh-cut cantaloupes under several commonly encountered temperature abuse scenarios, including chronic, acute, and intermittent temperature abuse conditions.

MATERIALS AND METHODS

Cantaloupes and sample preparation. Mature market grade cantaloupe fruits were purchased from local grocers and stored at 4°C overnight before use. Whole cantaloupes were immersed in distilled water containing 200 mg/liter of free chlorine (pH 6.5 adjusted using citric acid) for 2 min and rinsed in sterile distilled water. Washed cantaloupes were surface dried with paper towels and ventilation in a laminar flow hood for 30 min. After removal of seeds, rind (exocarp), and the innermost top layer soft tissue (endocarp), the cantaloupe flesh (mesocarp) was cut to cube-like pieces of ~8 cm³ and placed into 4-oz (112-g) portion cups for storage at 4°C until inoculation. Each sample was composed of three cubes that were from three different cantaloupes. Total weight of each sample was controlled at 25 ± 2 g.

Bacterial strains and inoculum preparation. Three *S. enterica* and three *L. monocytogenes* strains were used in this study as separate cocktail inoculums. Strains of *S. enterica* serovars Newport (USDA4558, mango isolate), Poona (FS3060, cantaloupe isolates), and Typhimurium (FDA1554, tomato isolate) and of *L. monocytogenes* (M101, serotype 4b, sausage isolate; M108, serotype 1/2b, salami isolate; and F6854, serotype 1/2a, frankfurter isolate) were all from the Environmental Microbiology and Food Safety Laboratory collections of the U.S. Department of Agriculture, Agricultural Research Service. Each of the strains was grown individually overnight in Luria-Bertani broth (BD, Sparks, MD) at 35°C with aeration. The overnight cultures were harvested by centrifugation and resuspended in equal volumes of phosphate-buffered saline (PBS; pH 7.2) and subsequently 1,000-fold diluted in PBS. Equal volumes of the three *S. enterica* (SE) or the three *L. monocytogenes* (LM) dilutions were mixed to obtain an SE or an LM cocktail as inoculum, of which each contained ~10⁶ CFU/ml of bacteria. For experiments testing the effect of native microflora (NM), washed *S. enterica* or *L. monocytogenes* cells were diluted into cell suspension (see below for preparation) of NM to obtain inoculum of SE-NM or LM-NM. *S. enterica* and *L. monocytogenes* cell counts in the inoculums were enumerated by plating on XLT-4 (Neogen, Lansing, MI) and Palcam (BD) agars, respectively.

NM preparation. Three whole cantaloupes were sequentially washed in 2 liters of PBS with brushing. The PBS wash solution containing bacteria from the cantaloupes was filtered through a 0.33-mm polyethylene filter in a filtered stomacher bag (Nasco, Salida, CA) and concentrated by centrifugation. After removal of coarse debris by low-speed centrifugation at 1,000 × *g* for 5 min, cells were precipitated by centrifugation at 5,000 × *g* for 15 min and resuspended in 1 ml of PBS. Cells were enumerated by plating on tryptic soy agar (BD), and a portion of the NM suspension was also plated on XLT-4 and on Palcam agars to screen for the presence of

Salmonella and *Listeria*, of which both were negative. Cells suspension were stored at 4°C and used within 24 h of preparation.

Inoculation of cantaloupe. Using an electronic repeater dispenser, each cantaloupe cube in the portion cups was inoculated by placing two 8.5-μl droplets of SE (or SE-NM) inoculum on two of the vertical facets and two 8.5-μl droplets of LM (or LM-NM) inoculum on the other two vertical facets, so that SE and LM inocula would not mix during the storage. As such, each sample (three cubes in a portion cup) was targeted to be inoculated with ~50 μl of SE and LM inocula, or ~5 × 10⁴ CFU per sample (or 2.0 × 10³ CFU/g) for *S. enterica* and *L. monocytogenes*. The coinoculated NM, when tested, was also controlled at ~5 × 10⁴ CFU per sample. The inoculated cantaloupe cubes were sealed in the portion cups and stored immediately at a specified temperature for a specified time. Noninoculated samples were also stored under the same conditions as the inoculated samples and were used for cantaloupe quality assessment.

Cantaloupe quality assessment. After storage at the designated temperature and time, noninoculated cantaloupe samples were visually inspected for quality deterioration by three laboratory personnel trained in produce quality evaluation on a 9-point hedonic scale, where 9 means “like extremely” and 1 means “dislike extremely” (19). Cantaloupe tissue integrity was assessed by measuring electrolyte leakage from the cantaloupe cubes following a modified method from Kou et al. (11). In brief, cantaloupe cubes in each portion cup were immersed in 25 ml of deionized distilled water for 30 min with intermittent shaking. The conductivity of the resultant solution was measured using an Accumet Basic AB30 conductivity meter (Fisher Scientific, Pittsburgh, PA). Cantaloupe cubes immersed in water were then frozen overnight at -20°C. After thawing at 37°C for 3 h, the conductivity of the water was similarly measured. The relative tissue electrolyte leakage was expressed as the ratio of prefreezing conductivity and postfreezing conductivity of the sample.

Microbiological analyses. After incubation at the designated temperature and time, inoculated cantaloupe cubes were immersed in 50 ml of SEL broth (9) and subjected to vigorous shaking by hand for 2 min. The wash solution filtered through a 0.33-mm polyethylene filter in a stomacher bag was 10-fold serially diluted in PBS, and appropriate dilutions were spiral plated on XLT-4 and Palcam agars in duplicates. After incubation at 35°C for 24 to 48 h, characteristic *S. enterica* and *L. monocytogenes* colonies on respective selective plates were counted, and cell populations on cantaloupe samples were calculated.

Experimental design and statistical analysis. The survival and growth of *S. enterica* and *L. monocytogenes* on fresh-cut cantaloupe subjected to various temperature abuses were compared with those of the control, in which no temperature abuse occurred. The experiment was conducted based on a factorial design with four replications. The *L. monocytogenes* and *Salmonella* populations were subjected to a log transformation before statistical analysis. Different samples were analyzed on each evaluation day for all studies. When effects were statistically significant, means were compared using the least significant difference test to maintain experiment-wise error *P* < 0.05.

RESULTS AND DISCUSSION

Temperature control is the most effective means of controlling growth of foodborne bacterial pathogens of perishable foods such as fresh produce. The U.S. Food and

Drug Administration (FDA) Uniform Food Code (28) requires all foods in the category of "Time and Temperature Control for Safety" be maintained at temperature not exceeding 5°C. Fresh-cut melons, tomatoes, and leafy green vegetables all belong to this category. However, incidences of violations of FDA recommendations occur, either due to the lack of proper equipment, improper employee training, or lack of understanding and awareness of the importance of temperature control in food safety. In this study, we examined several temperature abuse scenarios that potentially occur in the food supply chain as well as during consumer handling for the effect on the growth of foodborne bacterial pathogens on fresh-cut cantaloupe. *S. enterica* and *L. monocytogenes* were selected as they represent two different classes of foodborne bacterial pathogens for their ability of growth at low temperatures, and they have been historically associated with cantaloupe-related illness outbreaks.

Chronic temperature abuse. In this study, chronic temperature abuse was defined as sustained storage of time- and temperature-controlled food products at temperature exceeding that prescribed for safe storage (5°C). Chronic temperature abuses can occur at product distribution, retail display, and home storage when refrigeration operates at suboptimal conditions. Figure 1 shows the growth of *Salmonella* and *L. monocytogenes* in fresh-cut cantaloupe exposed to control (4°C) and abusive 8 and 12°C storage temperatures during a storage time of 1 week. At 4°C, *Salmonella* levels on fresh-cut cantaloupe stayed stable for the duration of the storage, whereas a steady increase in *Salmonella* populations was observed during the 8 and 12°C storage, with the populations reaching 4.32 and 5.16 log CFU/g, respectively, by the end of the week, or ~1.65 and 2.49 log units higher than that without temperature abuse. The *L. monocytogenes* populations steadily increased at all temperatures tested, reaching 4.44, 6.55, and 7.86 log CFU/g after 1-week storage at 4, 8, and 12°C, respectively, representing a proliferation by 0.75, 2.86, and 4.17 log units during the week-long storage at the respective temperatures.

Acute temperature abuse. This study defined acute temperature abuse as one-time, brief exposure of time- and temperature-controlled food products to room or higher temperatures. Two scenarios of acute temperature abuse were considered: exposure of fresh-cut cantaloupe to room temperature ($25 \pm 2^\circ\text{C}$) (mild) for 4 h and to 35°C (intense) for 2 h. Acute temperature abuses typically occur as consequence of delayed refrigeration after product processing or refrigeration failure afterward. These abuses are also likely to occur during shopping and at consumers' homes or after trips, and under some other scenarios. For *Salmonella*, exposure of inoculated cantaloupe cubes to a mild temperature abuse for 4 h on days 0, 2, and 7 postinoculation resulted in an increase of cell counts from 0.39 to 0.60 log unit, values comparable to those of cubes exposed to an intense temperature abuse for 2 h (0.32 and 0.54 log unit) (Table 1). The increase in *Salmonella* counts was comparable to the observation by Ukuku and Sapers

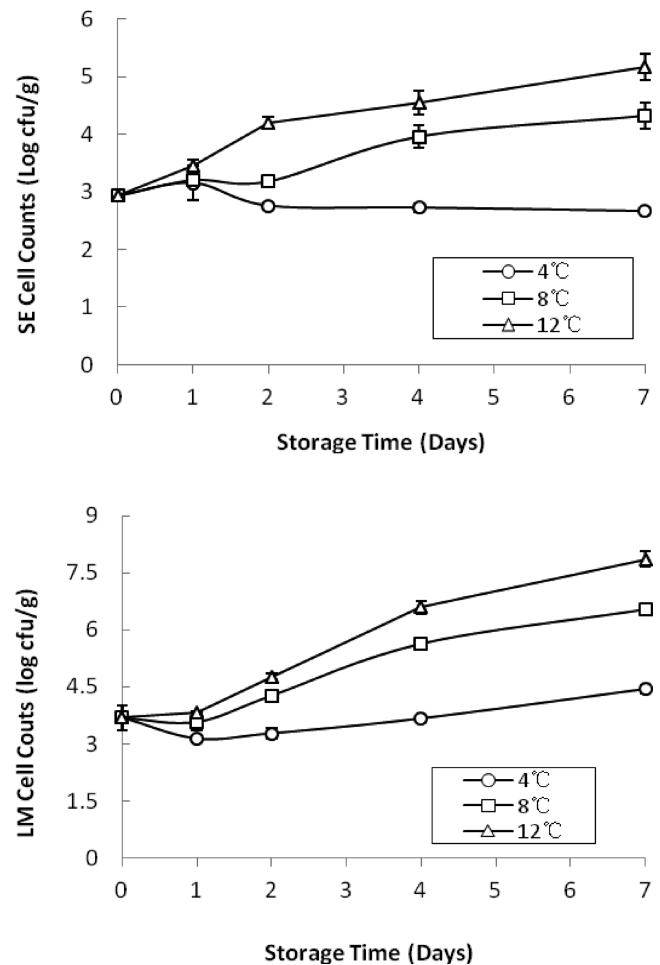


FIGURE 1. Growth of *S. enterica* (top) and *L. monocytogenes* (bottom) on fresh-cut cantaloupe affected by chronic temperature abuse.

(27) where inoculated *Salmonella* increased by ~1 log unit on inoculated fresh-cut cantaloupe that was held at 22°C for 5 h before refrigeration. For *L. monocytogenes*, the effect of exposure to acute temperature abuse was less significant, with *L. monocytogenes* populations increasing by 0.51 log unit for exposure to intense temperature abuse on day 2, and slightly decreasing for similar exposure on day 7 (Table 1). This was likely attributable to the relatively slow growth of *L. monocytogenes* compared with that of *Salmonella* at elevated temperatures that permit optimal growth of both pathogens.

Most acute temperature abuses are likely terminal, where products are either consumed or discarded after exposure to abusive temperatures. However, in some cases food subjected to acute temperature abuse could be returned to cold storage for consumption at later times. Therefore, we evaluated the growth potential of *Salmonella* and *L. monocytogenes* on fresh-cut cantaloupe during cold storage after being exposed to acute temperature abuse conditions (Fig. 2). Delaying refrigeration (4-h exposure to $25 \pm 2^\circ\text{C}$ before cold storage) after inoculation resulted in an increase in *Salmonella* population by 0.6 log unit, which was steadily maintained during the ensuing storage period. For *L. monocytogenes*, 4-h exposure to room temperature did not

TABLE 1. Effect of terminal acute temperature abuse on the growth of *S. enterica* and *L. monocytogenes* in the absence and presence of native microflora (NM)^a

Time	Acute temp abuse (without NM)			Acute temp abuse (with NM)		
	None	25 °C for 4 h	35 °C for 2 h	None	25 °C for 4 h	35 °C for 2 h
<i>Salmonella</i> (log CFU/g)						
Day 0	2.50 ± 0.15 A	3.10 ± 0.37 (0.60) ^b B	ND ^c	2.48 ± 0.22 A	3.07 ± 0.12 (0.59) B	ND
Day 2	2.85 ± 0.09 A	3.43 ± 0.33 (0.58) B	3.39 ± 0.32 (0.54) B	2.90 ± 0.10 A	3.26 ± 0.17 (0.36) B	3.29 ± 0.22 (0.39) B
Day 7	2.72 ± 0.07 A	3.11 ± 0.09 (0.39) B	3.04 ± 0.23 (0.32) B	2.64 ± 0.21 A	2.74 ± 0.19 (0.10) A	2.92 ± 0.07 (0.28) B
<i>L. monocytogenes</i> (log CFU/g)						
Day 0	2.71 ± 0.10 A	2.68 ± 0.18 (-0.03) A	ND	2.70 ± 0.10 A	2.76 ± 0.21 (0.06) A	ND
Day 2	3.28 ± 0.18 A	3.44 ± 0.17 (0.16) A	3.79 ± 0.17 (0.51) B	3.36 ± 0.22 A	3.43 ± 0.29 (0.07) A	3.57 ± 0.16 (0.21) B
Day 7	4.49 ± 0.13 A	4.75 ± 0.09 (0.26) B	4.34 ± 0.21 (-0.15) C	4.36 ± 0.06 A	4.79 ± 0.20 (0.43) B	4.19 ± 0.09 (-0.17) C

^a Values (means ± standard deviations) followed by different letters within a row (same day) for different temperature abuse conditions are significantly different at $P = 0.05$ according to the least significant difference test.

^b Number in parentheses after each cell count value indicates change in log unit in comparison to control (no temperature abuse).

^c ND, not determined.

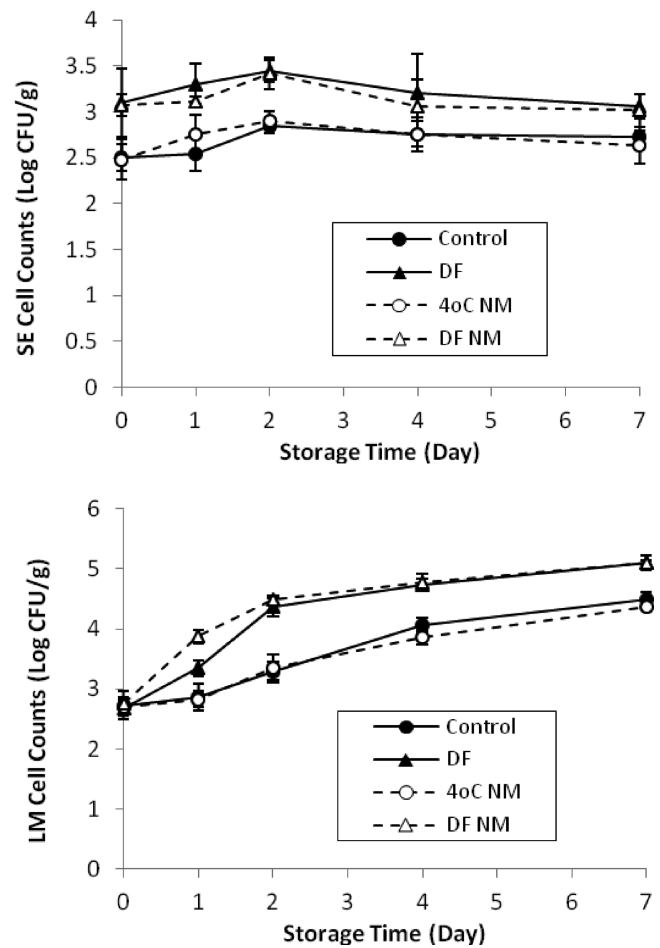


FIGURE 2. Growth of *S. enterica* (top) and *L. monocytogenes* (bottom) on fresh-cut cantaloupe affected by delayed refrigeration in the absence and presence of native microflora (NM). Inoculated cantaloupe cubes were stored at 4 °C (control) or subjected to delayed refrigeration (DF) by exposure to 25 °C for 4 h, followed by storage at 4 °C. Dashed lines indicate the presence of NM.

immediately result in a significant increase of cell counts on fresh-cut cantaloupe. However, the cell counts on the samples subjected to delayed refrigeration was ~0.5 log unit higher than the control when enumerated on the ensuing days, which was maintained during the week-long cold storage.

Intermittent temperature abuse. Intermittent temperature abuse was defined in this study as repeated occurrence of brief exposures to nonrefrigerating temperatures of time- and temperature-controlled foods. This is a less obvious but common form of temperature abuse, as exemplified by the suboptimally managed defrosting cycles at display cases in the grocery stores and other fresh-food holding entities. To assess the effect of the frequent temperature fluctuation on the survival and proliferation of foodborne bacterial pathogens, cold-stored (4 °C) fresh-cut cantaloupes inoculated with *Salmonella* and *L. monocytogenes* were exposed to two daily temperature shifts to 25 ± 2 °C for 15 min (Fig. 3). *Salmonella* populations steadily increased, reaching 1.95 log units higher than that of the control (4 °C, no temperature shift) at the end of a 7-day storage. The increase

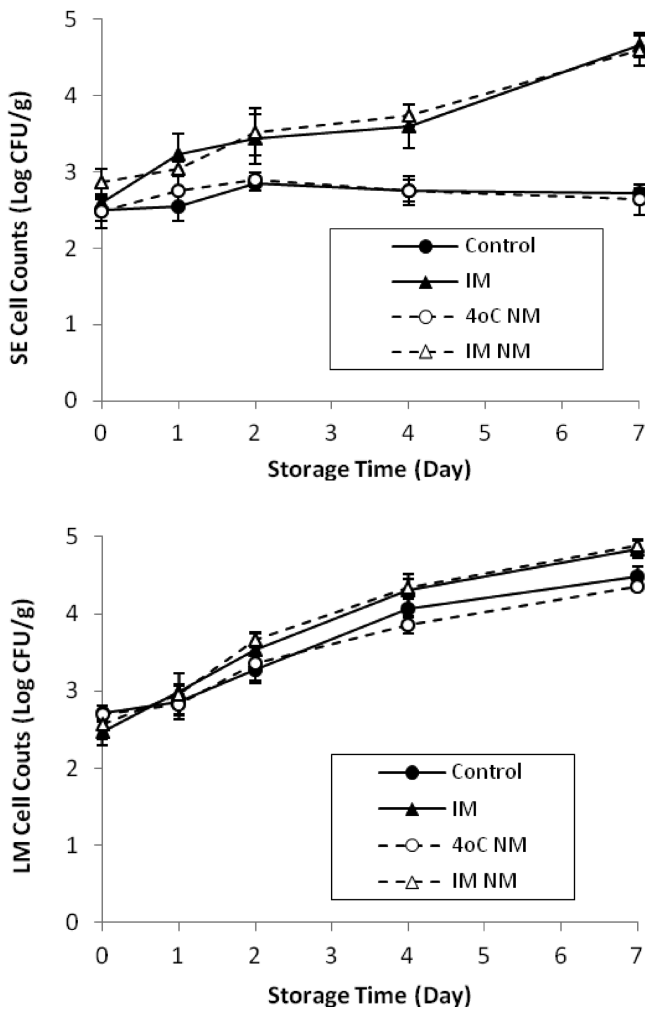


FIGURE 3. Growth of *S. enterica* (top) and *L. monocytogenes* (bottom) on fresh-cut cantaloupe affected by temperature fluctuation in the absence and presence of native microflora (NM). Inoculated cantaloupe cubes were stored at 4°C (control) or stored at 4°C with intermittent temperature abuse (IM) of two daily exposures to 25°C for 30 min. Dashed lines indicate the presence of NM.

was comparable to that for chronic temperature abuses at 8 and 12°C for 7 days. For *L. monocytogenes*, cell counts on samples subjected to intermittent temperature abuse increased by 2.38 log units at the end of the week-long storage, which was comparable to that observed for the chronic temperature abuse at 8°C. However, this increase was only 0.36 log unit higher than that without temperature abuse. The smaller increase of *L. monocytogenes* cell populations on fresh-cut cantaloupe subjected to frequent temperature fluctuation relative to constant storage at 4°C might be due to its ability of growth at refrigerating temperature as a psychrotroph.

Effect of NM. Several studies have demonstrated that the presence of NM significantly reduced the proliferation of bacterial pathogens on fresh produce (13, 26). At permissive temperatures, the competition for available nutrients and production of inhibitory metabolites by the NM significantly limit the growth of inoculated bacterial pathogens. In this study, the NM were recovered from the

rinds of cantaloupes and coinoculated with *Salmonella* and *L. monocytogenes* on fresh-cut cantaloupe (Table 1; Figs. 2 and 3). However, the survival and growth of the inoculated pathogens were not significantly affected by the presence of the NM under the tested temperature abuse conditions. This could be due to either the abundance of nutrients available on the surface of cut cantaloupe or to the total growth at the tested conditions being limited, which would not be sufficient for the manifestation of the underlying antagonistic interactions.

Effect of temperature abuse on quality of fresh-cut cantaloupe. Fresh-cut produce quality parameters, such as appearance, are often perceived as indicators of microbiological quality. However, several studies have shown that, under temperature abuse conditions, bacterial pathogens could grow to dangerous levels on fresh products without causing significant sensory or visual quality deterioration (15) aversive to consumers. Besides visual inspections by the researchers, the quality deterioration of the fresh-cut cantaloupe subjected to various temperature abuses was assessed by relative tissue electrolyte leakage (Table 2). Deterioration of appearance during the week-long storage at 4°C was not evident based on visual inspection, but relative tissue electrolyte leakage increased from 11.8 to 17.4%. Significantly more evident deterioration in visual quality of cut cantaloupe was observed by day 4 under all temperature abuse conditions, accompanied by more significant increase in relative tissue electrolyte leakage. Chronic temperature abuses at 12 and 8°C resulted in the most significant deterioration in cantaloupe quality, in terms of both visual scores and electrolyte leakage.

Data in this study showed that both *Salmonella* and *L. monocytogenes* were capable of significant growth on fresh-cut cantaloupe under various temperature abuse conditions. However, the proliferation of both pathogens were more significantly impacted by long-term suboptimal refrigeration (chronic temperature abuse) or frequent temperature fluctuation (intermittent temperature abuse) than short-term terminal exposure to higher temperatures (acute temperature abuse). These observations are in agreement with computer models predicting the growth of these pathogens at low and elevated temperatures (5, 12, 21, 22).

Current FDA food safety guidelines for time- and temperature-controlled foods recommend refrigeration at temperature not exceeding 5°C in supply chain, including food production, transportation, and retail. It is well recognized that temperature abuses in the food production–distribution chains can result in serious consequences to the well-being of consumers and economic health of the industry. Observations in this study strongly support the current FDA regulation on storage of time- and temperature-controlled foods, including fresh-cut cantaloupes. Household refrigeration is also an integral part of food safety paradigm. A national survey in 2006 found that in the United States, only 11% of household refrigerators had thermometers to monitor temperature and 34% of household refrigerators operated over the recommended safe setting of ~4°C (40°F) (10). In a small-scale survey in New Zealand,

TABLE 2. Quality deterioration of fresh-cut cantaloupe subjected to various temperature abuses^a

Storage (days)	Storage condition ^b :						
	4°C	8°C	12°C	IM	DF	TA 25°C	TA 35°C
Visual score (points)							
0	9	9	9	9	9	9	ND ^c
1	9	9	9	9	9	ND	ND
2	9 ^A	8.44 ± 0.39 ^{AB}	7.94 ± 0.85 ^B	8.11 ± 0.22 ^B	8.48 ± 0.48 ^{AB}	7.89 ± 0.22 ^{BC}	7.28 ± 0.26 ^C
4	8.67 ± 0.50 ^A	7.83 ± 0.66 ^B	6.56 ± 0.46 ^C	7.67 ± 0.56 ^B	7.56 ± 0.58 ^B	ND	ND
7	8.06 ± 0.17 ^A	5.06 ± 0.81 ^C	1.11 ± 0.33 ^D	5.72 ± 0.97 ^C	7.06 ± 0.17 ^B	5.94 ± 0.73 ^C	5.83 ± 0.71 ^C
Relative electrolyte leakage (%)							
0	11.79 ± 0.84 ^B	11.79 ± 0.84 ^B	11.79 ± 0.84 ^B	12.39 ± 1.16 ^{AB}	12.81 ± 0.21 ^A	12.39 ± 1.16 ^{AB}	ND
1	12.97 ± 1.77 ^B	14.41 ± 1.79 ^{AB}	15.93 ± 1.59 ^A	13.68 ± 0.48 ^B	13.21 ± 1.05 ^B	ND	ND
2	13.79 ± 0.53 ^C	17.06 ± 1.90 ^{AB}	18.28 ± 2.70 ^A	14.15 ± 0.85 ^C	14.58 ± 1.00 ^C	15.13 ± 3.75 ^{BC}	15.41 ± 0.84 ^{BC}
4	16.45 ± 0.70 ^B	18.48 ± 1.59 ^A	19.91 ± 1.49 ^A	18.48 ± 2.26 ^A	18.34 ± 1.95 ^{AB}	ND	ND
7	17.42 ± 1.28 ^E	21.68 ± 1.03 ^B	24.31 ± 1.10 ^A	18.65 ± 0.72 ^{CD}	19.10 ± 1.08 ^C	17.68 ± 0.56 ^{DE}	17.71 ± 0.67 ^{DE}

^a Values (means ± standard deviations) followed by different letters within a row (same day) for different temperature abuse conditions are significantly different at $P = 0.05$ according to the least significant difference test.

^b Storage conditions (from left to right) are as follows: control, 4°C; chronic temperature abuse, 8°C; chronic temperature abuse, 12°C; intermittent (IM) temperature abuse, 4°C with two 15-min daily exposures to 25°C; delayed refrigeration (DF), 4-h exposure to 25°C before storage at 4°C; terminal acute (TA) temperature abuse, 4°C followed by 4-h exposure to 25°C before sampling; and TA temperature abuse, 4°C followed by 2-h exposure to 35°C before sampling.

^c ND, not determined.

13 to 36% of household refrigerators operated above 6°C at different times of the day (20). Therefore, stronger enforcement of regulations for preventing temperature abuse need to be matched with heightened efforts of consumer education to minimize postproduction proliferation by foodborne pathogens in time- and temperature-controlled foods.

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