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Growth and survival of *Salmonella enterica* and *Listeria monocytogenes* on fresh-cut produce and their juice extracts: Impacts and interactions of food matrices and temperature abuse conditions

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ABSTRACT

Storage temperature and nutrient availability are major factors impacting pathogen growth and thus food safety risks. This study evaluated the survival and growth of *Salmonella enterica*, and *Listeria monocytogenes* in relation to temperature abuse variations, and food matrices. Fresh-cut cantaloupe, honeydew, watermelon, pineapple, and radish contaminated with *S. enterica* and *L. monocytogenes* were subjected to cold (4 °C), chronic temperature abuse at 8 and 12 °C, and acute temperature abuse (35 °C for 2 h followed by 4 °C for the remainder 7-day storage). Pathogen growth potential in the juice extracts from each product was further compared to that on the respective cut produce. Under chronic temperature abuse, three different pathogen growth patterns emerged on five test products: both *S. enterica* and *L. monocytogenes* grew significantly on cut cantaloupe, honeydew and watermelon at 8 and 12 °C; but only survived on cut radish, and even declined in population on cut pineapple under the same conditions. Specifically, *S. enterica* populations reached up to 5.28 log CFU/g and *L. monocytogenes* up to 7.77 log CFU/g after 7 days at 12 °C. During cold storage at 4 °C, significantly different growth patterns were also observed between *S. enterica* and *L. monocytogenes* on cut melons, where *S. enterica* populations remained unchanged during the 7-day storage while *L. monocytogenes* grew continuously. In the juice extracts, *S. enterica* and *L. monocytogenes* reached maximum population density in melon juices, but failed to grow in pineapple juice, similar to the growth patterns on cut melon and pineapple. Distinctly different growth patterns, however, were shown in *S. enterica* and *L. monocytogenes* on cut radish and in radish juice; exhibiting no growth on cut radish, but maximum growth in radish juice. The disparity in pathogen growth observed on cut pineapple and radish versus on melon in this study supports commodity specific risk-based food safety policies pertaining to temperature control for food safety.

1. Introduction

Fruits and vegetables are rich sources of nutrients, vitamins, and dietary fiber, and are important components of a healthy diet. However, the number of foodborne outbreaks associated with consumption of contaminated fresh and fresh cut produce has increased in recent years (CDC, 2018a; Sivapalasingam, Friedman, Cohen, & Tauxe, 2004). Among different produce commodity groups, vine-stalk vegetables accounted for the most illnesses (12%), followed by fruits/nuts (6.3%), leafy vegetables (5.2%), and root vegetables (2.7%) (Painter et al., 2013). Several major outbreaks of *S. enterica* and *L. monocytogenes* have been traced back to contaminated fruit, especially melons including cantaloupe, honeydew and watermelon (Walsh, Bennett, Mahovic, & Gould, 2014). For example, in 2018, 77 individuals were sickened in a multistate outbreak of *Salmonella* Adelaide infections linked to consumption of fresh-cut melon (CDC 2018b). In 2011, contaminated cantaloupe was the source of a multistate outbreak of *L. monocytogenes* infections that sickened at least 147 people in 28 states (CDC, 2012).

Many microorganisms are indigenous to fresh and fresh-cut produce and contamination by foodborne pathogens can occur at various points in the farm to fork continuum (Beuchat, 1996; Hanning, Nutt, & Ricke, 2009). Microbial contaminants can be introduced onto melons from multiple sources including irrigation water, air borne particles, wash water, and packing facilities (Gagliardi, Millner, Lester, & Ingram,

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2003) and can be transfererred from rind to flesh during cutting (Shearer, LeStrange, Castaneda Saldana, & Kniel, 2016). The current U.S. Food Code requires that Time/Temperature Control for Safety (TCS) foods, including fresh-cut melon, tomato, and leafy green vegetables, be maintained at 5 °C or below (FDA, 2017). These requirements for time/temperature control are primarily determined by the potential for pathogenic microorganisms of concern to contaminate, survive, and subsequently grow and/or produce toxin on a given commodity (Jol, Kassianenko, Wszol, & Oggel, 2006).

Temperature abuse can occur at different stages of food processing, storage, and distribution. Chronic temperature abuse occurs when TCS foods are stored at sustained temperatures exceeding the limit for safe storage (5 °C). This type of temperature abuse typically occurs during product distribution, retail display, or home storage, when produce in transit or storage is refrigerated at suboptimal conditions. A survey of domestic and commercial refrigerators in the US in 2006 found that 20% of equipment were operated at > 10 °C (Jol et al., 2006). Recent studies from Zeng et al. (2014) also reported that 30% of temperatures recorded in an open case at the retail level were above 5 °C. Another type of frequently occurring temperature abuse is represented by one-time exposure of TCS foods to room or higher temperatures. Such acute temperature abuse is usually the consequence of delayed refrigeration after product processing or refrigeration failure at some point prior to consumption, as well as food products left at ambient temperature for an extended period at consumer homes.

Survival and proliferation of pathogens on produce is significantly enhanced on fresh-cut produce due to the nutrients released from the cut surface (Heaton & Jones, 2008). Since melons are typically rich in sugars and other nutrients and have a near neutral pH, *S. enterica* and other pathogens can grow significantly if fresh-cut melon is contaminated and improperly stored. While the general effect of food matrices on pathogen growth is established, specific information is lacking regarding pathogen multiplication on different fruit and vegetable surfaces and especially their different responses to temperature abuse. These important data gaps significantly hinder the development and implementation of science- and risk-based food safety regulations. Thus, the main objectives of this study were to 1) examine the effect of chronic and acute temperature abuse scenarios on the survival and growth of *S. enterica* and *L. monocytogenes* on three types of fresh-cut melon, as well as on pineapple, and radish; and 2) explore the differential response of pathogen growth on cut surfaces and in juice extract as impacted by fresh-cut produce matrices with similar, and distinct texture and chemical compositions.

2. Materials and methods

2.1. Sample preparation

Mature, market-grade cantaloupes, honeydews, watermelons, pineapples, and radishes were purchased from local wholesale market and stored at 4 °C overnight before use. The whole fruits and radishes were then immersed in a wash solution containing 200 mg/L of free chlorine (adjusted to pH 6.5 using citric acid) for 2 min and rinsed in sterile distilled water. Washed fruits and radishes were surface-dried with paper towels followed by air drying in a laminar flow hood for 30 min. The samples were prepared by removing the seeds, rind, and innermost top layer of soft tissue of the melons, or the peels of pineapples and radishes. The mesocarp of the melons, and the flesh of the pineapples and radishes were cut into cuboid pieces of approximately 8 cm^3, placed into 4 oz (~120 cm^3) portion cups, and kept at 4 °C until inoculation. Each sample cup contained three sample pieces taken from three separate fruits or vegetables of the same kind.

2.2. Bacterial strains and inoculum preparation

The fruit and radish samples were inoculated with a cocktail of three *S. enterica* (SE) serovars: Newport (USDA4558, mango isolate), Poona (FS3060, cantaloupe isolates), and Typhimurium (FDA1554, tomato isolate), and a *L. monocytogenes* (LM) cocktail consisting of three serotypes of *L. monocytogenes:* serotypes 4b (M101, sausage isolate), 1/2b (M108, salami isolate), and 1/2a (F6854, frankfurter isolate). All strains were obtained from USDA-ARS Environmental Microbiology and Food Safety Laboratory (EMFSL) collections. Each of these six strains were grown individually overnight in Luria-Bertani (LB) broth (Becton, Dickinson and Co., Sparks, MD) at 35 °C with aeration. Overnight cultures were harvested by centrifugation, and re-suspended in equal volumes of phosphate-buffered saline (PBS). After a 1000-fold dilution in PBS, each of the three *S. enterica* or *L. monocytogenes* cell suspensions were pooled in equal volumes to obtain a *S. enterica* or a *L. monocytogenes* cocktail for use as an inoculum approximating 10^6 CFU/ml. The concentrations of *S. enterica* and *L. monocytogenes* cocktails were determined by plating on XLT-4 (Neogen, Lansing, MI) and Palcam (Becton, Dickinson and Co.) agars, respectively.

2.3. Inoculation and storage conditions

Cubes for each food matrix were inoculated with two droplets of *S. enterica* inoculum (8.5μl/drop) on two of the vertical facets and two droplets of *L. monocytogenes* inoculum (8.5μl/drop) on the other two vertical facets to avoid mixing the two inocula during storage. As such, each sample (three cubes in one portion cup) was inoculated with approximately 50 μl of *S. enterica* and *L. monocytogenes* inoculum, respectively, which approximately represents 2.0×10^3 CFU/g for each of the cocktails. The inoculated cubes and un-inoculated control cubes were then sealed in the portion cups and stored immediately at a specified temperature for a specified duration as follows: (a) 4 °C for 7 days; (b) 8 °C for 7 days (chronic temperature abuse at 8 °C); (c) 12 °C for 7 days (chronic temperature abuse at 12 °C); and (d) 35 °C for 2 h followed by 4 °C for 7 days.

2.4. Fruit and vegetable juice extraction

Juices from each of the fruits and vegetables used in this study were extracted using a household juice extractor, and filtered through a Whirl-Pak filter bag (Nasco, Ft. Atkinson, WI) with 0.33 mm perforation. These filtered juices were then centrifuged at 4000 rpm for 10 min. The supernatant was filtered through a 0.22 μm nylon filter and used as growth media for determining growth profiles of *S. enterica* and *L. monocytogenes* strains.

2.5. Growth monitoring in fruit and vegetable juices

In a 96-well plate, 200 μl of each type of sterile filtered juice and tryptic soy broth was mixed with 1 μl of *S. enterica* or *L. monocytogenes* inoculum in 12 replicates. Plates were incubated at 37 °C with shaking and the growth of the inoculated bacteria was monitored by measuring the absorbance at 600 nm. The absorbance was measured at 20 min and 30 min intervals for *S. enterica* and *L. monocytogenes*, respectively, over 18–24 h. The optical density readings (OD_{600}) were converted to bacterial cell populations based on standard curves established for each corresponding food matrix and pathogen pair. Each growth curve was further modeled using USDA Integrated Pathogen Modeling Program (IMPI 2013) to derive the growth parameters according to Huang (2008 and 2013). These include lag phase duration (λ), specific growth rate (μ_{max}), and maximum bacterial population (Υ_{max}).

2.6. Microbial enumeration

The inoculated and un-inoculated cubes from each sample were aseptically removed from the portion cups on days 0, 1, 2, 4, and 7, and transferred into individual sterile Whirl-Pak filter bags (Fort Atkinson, WI). SEL broth *(25)* (50 ml) was added to each sample bag, which was

then vigorously shaken by hand for 2 min, and appropriate dilutions of the filtrate were spiral plated on XLT-4 and Palcam agars in duplicate. After 24–48 h incubation at 35 °C, typical *S. enterica* colonies (i.e., pink-red with a black center and yellow periphery) were counted on the XLT-4 plates, and typical *L. monocytogenes* colonies (i.e., gray-green colonies surrounded by dark brown to black halos in the medium) were counted on the Palcam plates.

2.7. Statistical analysis

Microbial data were log transformed and analyzed using PROC MEANS for univariate statistics (SAS Institute Inc 1999, Cary, NC). Additionally, the data were analyzed according to a two-factor (commodity type and temperature) linear model using the PROC MIXED procedure (SAS Institute Inc 1999, Cary, NC). Assumptions of normality and variance homogeneity of the mixed model were checked and the variance grouping technique was used to correct for variance heterogeneity. When effects were statistically significant, means were compared using Sidak adjusted p-values to maintain experiment-wise error ≤ 0.05. Treatment differences were tested with Tukey-Kramer test at $\alpha = 0.05$ and interactions were tested as well. Unless otherwise indicated, only treatment differences at $\alpha = 0.05$ were discussed.

3. Results

3.1. Growth and survival of S. enterica on fresh-cut fruit and radish under cold, and chronic and acute temperature abuse conditions

Fresh-cut fruit and radish inoculated with *S. enterica* were stored under conditions of cold (4 °C consistently for 7 days), chronic temperature abuse at 8 °C or 12 °C for 7 days, and acute temperature abuse (2 h at 35 °C followed by 7 days at 4 °C). Fig. 1A shows that *S. enterica* counts persisted at around 2.6 log CFU/g, with slight decrease for the duration of the experiment for all types of fresh-cut fruit and radish except for pineapple. On the contrary, *S. enterica* counts on cut pineapple exhibited a steep decline within the first 2 days, from 2.6 log CFU/g on day 0–1.76 log CFU/g on day 1 to 0.22 log CFU/g on day 2. This low level was sustained for the duration of the remaining storage.

Under chronic temperature abuse conditions, *S. enterica* populations increased significantly on cut cantaloupe, honeydew and watermelon ($p < 0.05$), reaching up to 4.38 and 5.28 log CFU/g, for 8 °C (Figs. 1B) and 12 °C (Fig. 1C), respectively. In contrast, *S. enterica* population on cut pineapple steadily declined during the storage at both 8 °C and

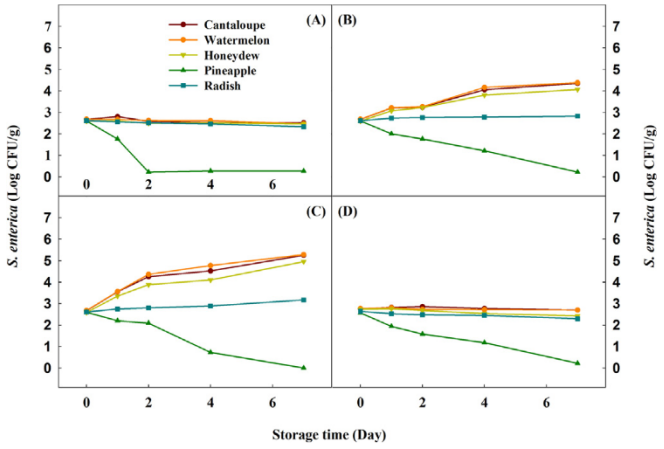

Fig. 1. Growth of *Salmonella enterica* on fresh-cut cantaloupe, honeydew, watermelon, pineapple, and radish stored under different temperature condition. (A) 4 °C for 7 days; (B) 8 °C for 7 days; (C) 12 °C for 7 days; and (D) 35 °C for 2 h followed by 4 °C for 7 days. Data represent the mean of three replications. The lower limit of detection is 5 CFU/g.

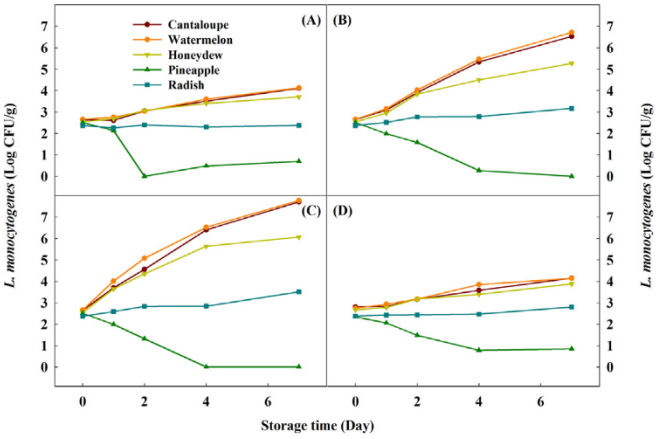

Fig. 2. Growth of *Listeria monocytogenes* on fresh-cut cantaloupe, honeydew, watermelon, pineapple, and radish stored under different temperature condition. (A) 4 °C for 7 days; (B) 8 °C for 7 days; (C) 12 °C for 7 days; and (D) 35 °C for 2 h followed by 4 °C for 7 days. Data represent the mean of three replications. The lower limit of detection is 5 CFU/g.

12 °C, dropping to ca. 0.22 log CFU/g or lower by the end of 7-day storage. *S. enterica* counts on cut radish stayed at ca. 2.7 log CFU/g during the entire storage time at 8 °C, with a slight increase from ca. 2.7 log CFU/g to ca. 3.2 log CUF/g by the end of 7-day storage at 12 °C.

In the acute temperature abuse scenario, where the cut products were briefly exposed to high temperature (35 °C for 2 h) before resuming refrigeration at 4 °C for the remainder of the 7-day storage, the growth of *S. enterica* on the cut fruit and radishes was comparable to that under consistent 4 °C storage (Fig. 1D). However, for the cut pineapple, the rate at which the *S. enterica* population dropped was slower compared to those without this acute temperature abuse.

3.2. Growth and survival of L. monocytogenes on fresh-cut fruits and radish under cold, and chronic and acute temperature abuse conditions

L. monocytogenes populations on fresh-cut cantaloupe, honeydew and watermelon were stable for the first day, and increased slowly but steadily afterward, from 2.5 to 2.6 log CFU/g on day 0–3.7–4.1 log CFU/g by day 7, which represents significant growth (Fig. 2A). *L. monocytogenes* populations on cut radish remained relatively stable throughout the 7-day storage at 4 °C (ca. 2.3 log CFU/g). On the contrary, *L. monocytogenes* populations on cut pineapple stored at 4 °C decreased significantly within the first 2 days from 2.51 log CFU/g on day 0 to nearly undetectable levels on day 2. Subsequently, *L. monocytogenes* populations increased gradually to 0.7 log CFU/g by the end of the storage period.

Under chronic temperature abuse, *L. monocytogenes* populations on melon rapidly increased from 2.5 log CFU/g to as high as 6.72 and 7.77 log CFU/g at 8 °C and 12 °C, respectively over the 7-day storage period (Fig. 2B and C). *L. monocytogenes populations* on radish had minimal changes under these temperature abuse conditions. In contrast, *L. monocytogenes* populations on cut pineapple steadily and rapidly declined through the first 4 days of storage at these elevated temperatures, until they were below the detection limit.

As with *S. enterica*, the *L. monocytogenes* populations showed similar growth patterns on cut fruit and radish subjected to acute temperature abuse. Slight increase in *L. monocytogenes* populations was observed on cut melon, while no growth was seen on cut radish (Fig. 2D). Although there was a continuous decline in *L. monocytogenes* populations on cut pineapple subjected to the acute temperature abuse scenario, the decrease in population occurred more slowly on these samples than on pineapple control samples (without the 2-h exposure to 35 °C).

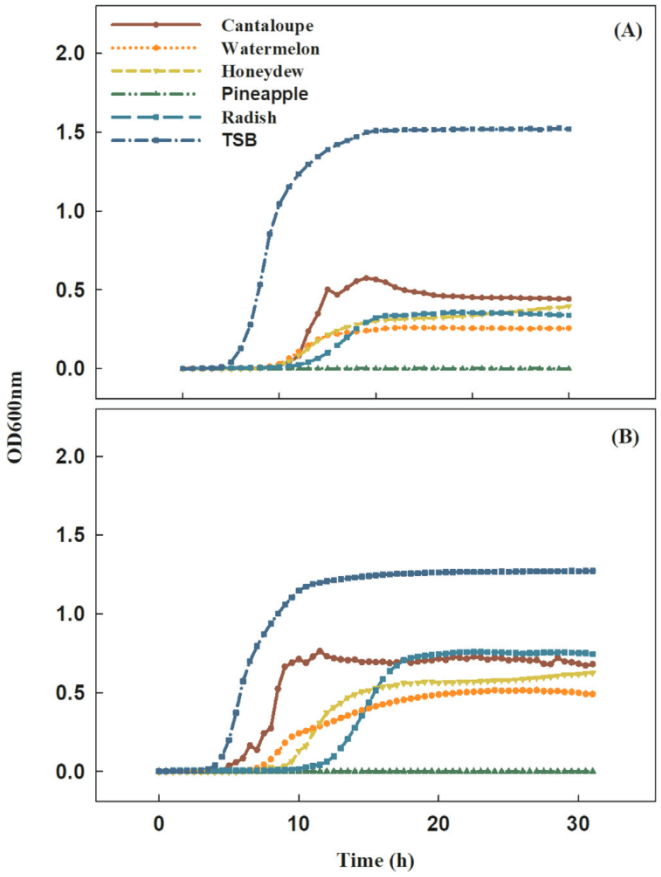

Fig. 3. Growth kinetics of *Salmonella enterica* (A) and *Listeria monocytogenes* (B) in juices prepared from fresh-cut fruits and radish. Data shown represent the mean of 12 replicates in the experiment.

3.3. Growth kinetics of S. enterica and L. monocytogenes in juice extracts

To assess the potential mechanism behind the three distinctive growth patterns of these five fresh-cut products, i.e. significant growth on melon, no growth on radish, and decline on pineapple, additional studies were conducted concerning growth potential in the juice extracts of the respective produce. As shown in Fig. 3, juice of all melon types supported growth of both *S. enterica* (Fig. 3A) and *L. monocytogenes* (Fig. 3B). Among all three melons, the growth for both pathogens in cantaloupe juice had the shortest lag phase and reached the highest growth potential as observed with the highest OD600 values (Table 1). Pathogen growth in radish juice showed longer lag phase than that in melon juices, but nevertheless reached similar cell counts as those in melon juices quickly. On the other hand, pineapple juice completely inhibited the growth of *S. enterica* and *L. monocytogenes*.

4. Discussion

Temperature is one of the most important factors affecting cellular metabolic reactions (Francis et al., 2012; Garcia-Gimeno, Castillejo-Rodriguez, Barco-Alcala, & Zurera-Cosano, 1998; Kou et al., 2014). It is also the critical factor determining the survival and growth of pathogens on various food matrices, including fresh-cut produce (Francis O'Beirne, 2001; Huang, Luo, & Nou, 2015; Sudarshana, Bandyopadhyay, Rosa, Suslow, & Harris, 2008; Luo, He, McEvoy, & Conway, 2009; Luo, He, & McEvoy, 2010). Therefore, consistently maintaining adequate refrigeration during the transportation and storage of fresh-cut produce is a critically important practice to uphold produce quality and safety. The recently issued Sanitary Transportation Rule for Human Foods under Food Safety Modernization Act (FSMA), emphasizes temperature control as a critical element to ensure food safety.

Table 1
Key nutrient characteristics of juice extracts from each food matrix and the growth potentials of *S. enterica (SE)* and *L. monocytogenes (LM)*.

Medium	pH	Sugar content (%Brix)	Growth potential for *SE*[a]			Growth potential for *LM*		
			λ (h)[b]	μ_{max} (1/h)	Y_{max} (log cfu/ml)	λ (h)	μ_{max} (1/h)	Y_{max} (log cfu/ml)
Tryptic soy broth	7.30	2.5	0.45	1.03	9.16	2.17	0.70	9.09
Cantaloupe juice	6.47	9.6	2.76	0.64	9.16	2.95	0.59	9.28
Honeydew juice	5.81	6.8	3.23	0.97	8.51	5.25	0.42	8.76
Watermelon juice	5.41	7.9	3.21	0.58	8.75	4.12	0.57	8.46
Radish Juice	5.95	4.3	4.54	0.46	8.62	8.61	0.33	9.36
Pineapple Juice	3.34	13.4	N/A	0	5.59	N/A	0.00	6.09

[a] Bacterial cultures were incubated at 37 °C for up to 24 h.
[b] λ is the lag phase duration; μ_{max} is the specific growth rate (maximum growth rate); Y_{max} is the maximum bacterial population. These three growth parameters were derived via USDA Integrated Pathogen Modeling Program described in detail by Huang (2008, 2013).

Previous studies on foodborne pathogens such as *Escherichia coli* O157:H7, *S. enterica* and *L. monocytogenes* have evaluated their growth under temperature abuse conditions on many fruits and vegetables such as lettuce (Koseki & Isobe, 2005), pepper and tomato (Ma, Zhang, Gerner-Smidt, Tauxe, & Doyle, 2010), radish (Islam et al., 2004), cut collard greens (Sant'Ana, Barbosa, Destro, Landgraf, & Franco, 2012), melon and pineapple (Abadias, Alegre, Oliveira, Altisent, & Vinas, 2012; Danyluk, Friedrich, & Schaffner, 2014), cantaloupe and honeydew (Hong, Yoon, Huang, & Yuk, 2014; Nyarko et al., 2016a; 2016b). These studies mainly examined the survival and growth of these pathogens during storage at constant temperatures, such as at 4 °C, 12 °C, 20 °C, or 25 °C, or on one type of cut produce. In this study, we examined the survival and growth of *S. enterica* and *L. monocytogenes* on five types of cut produce under various temperature abuse scenarios that may be encountered in the supply chain due to lack of proper temperature management, or during equipment failure. Presently, the US Food Code requires time and temperature control for safety foods be maintained at 5 °C or below (FDA, 2017). This includes fresh-cut cantaloupe, honeydew, and watermelon. In this study, the significant growth of pathogens that we observed on cut melon during chronic temperature abuse further confirms the critical importance of temperature control and compliance to US Food Code to mitigate pathogen growth on cut melon. It is worth noting that while *S. enterica* exhibited no growth at 4 °C, *L. monocytogenes* showed slow, but continuous, growth at this cold temperature. This suggests that increased *L. monocytogenes* risks exist on certain food products during prolonged storage even at cold storage temperature.

Drastically different pathogen growth patterns were observed on cut melon, pineapple, and radish in response to temperature abuse, and in their juice extracts. In general, both *S. enterica* and *L. monocytogenes* population increased significantly during chronic temperature abuse on cut melon, and in melon juice extracts. With high sugar content and relatively low acidity, melon juices are well suited for supporting the growth of bacterial pathogens such as *S. enterica* and *L. monocytogenes*, at permissive temperatures. On the other hand, both *S. enterica* and *L. monocytogens* declined on pineapple cut surfaces and there was no growth in pineapple juice, suggesting that chemical properties of pineapple juice may have played a key role in inhibiting pathogen growth. Pineapple juice has high acidity with a pH around 3.2–4.0 (Abadias et al., 2012), which is well below the minimum pH for either *S. enterica* or *L. monocytogenes* to grow (Foster & Spector, 1995; Tienungoon, Ratkowsky, McMeekin, & Ross, 2000). Overall, the

consistent results on cut fruit surfaces and in juices from all three types of melon and pineapple are in line with the theory that the growth capacity of bacteria on a food matrix depends on the physiochemical properties of food products.

Contrary to melon and pineapple, cut radish and radish juice had completely different effects on pathogen growth, i.e. cut radish restricted the growth of *S. enterica* and *L. monocytogenes,* while radish juice supported full growth potential of both pathogens. Studies from Iványovics and Horváth (1947) and Shukla, Chatterji, Yadav, and Watal (2011) showed anti-bacterial effect from raphanin in radish. This may explain the longer lag phase of *S. enterica* and *L. monocytogenes* had in radish juice extract. Neverthelss, no significant difference was observed in the maximum bacterial population density in either radish juice or melon juice. The disparate pathogen growth pattern on radish cut surface and in radish juice suggests that something else, other than the nutrient content of radishes, may have played a significant role in restricting pathogen growth. Visual observation of the tested products indicate that melon and pineapple have soft cut-surface textures with juice and nutrients readily released during and after cutting, while the firm texture of radish does not easily release nutrients or juice under the same conditions. Thus, it is plausible that reduced availability of nutrient from the cut radish tissues may have restricted the pathogen growth on the radish cut surface. Future studies are needed to explore the impact of chemical, physical, and physiological factors of fresh produce on pathogen growth.

5. Conclusion

This study evaluated the growth trend of *Salmonella enterica* and *Listeria monocytogenes* on five produce matrices under four storage temperature regimes, along with pathogen growth potential in juice extracts of these produce commodities. Results indicated that pathogen growth is dependent on pathogen type, produce matrices, and storage temperature. Findings highlight the importance of risk-based commodity-specific food safety polices regarding temperature control. Results may also provide insight for produce breeding programs and fresh-cut processing innovations to limit pathogen growth.

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