



Fate of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* on fresh-cut celery

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ABSTRACT

Illnesses from *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* have been associated with the consumption of numerous produce items. Little is known about the effect of consumer handling practices on the fate of these pathogens on celery. The objective of this study was to determine pathogen behavior at different temperatures under different storage conditions. Commercial fresh-cut celery was inoculated at ca. 3 log CFU/g onto either freshly cut or outer uncut surfaces and stored in either sealed polyethylene bags or closed containers. Samples were enumerated following storage for 0, 1, 3, 5, and 7 days when held at 4 °C or 12 °C, and after 0, 8, and 17 h, and 1, and 2 days when held at 22 °C. At 4 °C, all populations declined by 0.5–1.0 log CFU/g over 7 days. At 12 °C, *E. coli* O157:H7 and *Salmonella* populations did not change, while *L. monocytogenes* populations increased by ca. 0.5 log CFU/g over 7 days. At 22 °C, *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* populations increased by ca. 1, 2, or 0.3 log CFU/g, respectively, with the majority of growth occurring during the first 17 h. On occasion, populations on cut surfaces were significantly higher than those on uncut surfaces. Results indicate that populations are reduced under refrigeration, but survive and may grow at elevated temperatures.

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1. Introduction

Celery is commonly consumed in two manners, either as an ingredient or raw, as a snack. The value-added segments of fresh-cut celery sticks are gaining in popularity as part of the growing market for washed, ready-to-eat produce products (Lucier and Jerardo, 2005). Celery has been associated with some documented outbreaks of foodborne disease. In 1899, an outbreak of typhoid fever at a Massachusetts asylum resulting in 40 ill inmates over a 2-week period was linked to celery consumption (Morse, 1899). An outbreak of acute gastroenteritis amongst approximately 1440 cadets at the U.S. Air Force Academy in the early 1990's was linked to the consumption of chicken salad. Originally attributed to *Salmonella*, celery exposed to norovirus in nonpotable water and used as an ingredient in the chicken salad was eventually identified as the cause (Warner et al., 1991). In 2010, diced celery,

used as ingredient in chicken salad in a hospital, was the cause of a listeriosis outbreak in Texas that led to ten confirmed cases and five deaths in immunocompromised, hospitalized patients. The outbreak strain was isolated from chicken salad, the diced celery ingredient at the hospital and in 19 of >200 surface swabs and 8 of 11 diced celery products at the processing plant (Gaul et al., 2013; Texas DSHS, 2010; United States Food and Drug Administration (US FDA), 2010).

Although no *Escherichia coli* O157:H7 outbreaks have been associated with fresh-cut celery, this organism has been isolated from 23/89 (27%) vegetable samples (including celery) in Mexico (Zepeda-Lopez et al., 1995). *Listeria monocytogenes* was isolated from one unprocessed raw celery sample at a health-care food service location in Ontario, Canada (Odumeru et al., 1997), and in 2 of 13 (15.4%) 25 g samples of fresh-cut celery taken from supermarket-prepared salads in Chile (Cordano and Jacquet, 2009). *Salmonella* was isolated from 2 of 26 (8%) 100-g celery samples obtained from the central market of Granada, Spain (Garcia-Villanova et al., 1987), 5 of 8 (63%) 25-g celery samples from street vendors in Mumbai, India (Viswanathan and Kaur, 2001) and 3 of 100 (3%) 50-g celery samples from a central supply station in Mexico City (Quiroz-Santiago et al., 2009).

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Fresh-cut celery is considered to be ready-to-eat without any further preparation by the consumer. Risks from fresh-cut celery consumption may increase from temperature abuse leading to multiplication of pathogens. Data on the survival and growth of pathogens are essentially non-existent for celery, and the influence of consumer handling contribution to risks associated with consumption of fresh-cut celery is unknown. Our objective was to quantify the impacts of time and temperature, and storage container on fate of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* on fresh-cut celery under simulated consumer storage conditions.

2. Materials and methods

2.1. Fresh-cut celery

Bags of fresh-cut celery advertised as “washed and ready-to-eat,” were purchased from a local supermarket (Winter Haven, FL). Celery was purchased within one day of store delivery and held at 4 ± 2 °C for up to 24 h prior to use in experiments. Immediately prior to inoculation, celery sticks were cut to 10 ± 1 g pieces using a sterile, serrated knife and cutting board.

2.2. Strain selection

Cocktails of strains, from produce outbreaks when available, were used as inoculum in all experiments. Five strains of *E. coli* O157:H7 were selected: H1730 (lettuce outbreak, human feces), F4546 (alfalfa sprout outbreak, human feces), 223 (Odwalla apple juice outbreak), EC4042 (spinach outbreak), and F658 (cantaloupe outbreak, human feces). Four strains of *L. monocytogenes* were selected: LCDC 81-861 (raw cabbage outbreak), Scott A (perinatally transmitted human milk outbreak), 101M (beef outbreak), and V7 (cow milk outbreak). Five serotypes of *Salmonella* were selected: Michigan (LJH 521, cantaloupe outbreak; human feces), Montevideo (G4639, tomato outbreak; human feces), Enteritidis phage type 30 (ATCC BAA 1045, raw almond outbreak; human feces), Agona (LJH 517, alfalfa sprout outbreak; human feces), and Gamilara (F2712, orange juice outbreak; human feces). Strains were adapted to grow in the presence of rifampicin or nalidixic acid through the use of a stepwise exposure to increasing concentrations of antibiotic (Parnell et al., 2005); *E. coli* O157:H7 and *Salmonella* were made resistant to 80 µg/ml rifampicin (Fisher Bioreagents, Fair Lawn, NJ), and *L. monocytogenes* to 50 µg/ml nalidixic acid (Sigma Aldrich, St. Louis, MO).

2.3. Inoculum preparation

Prior to each experiment, frozen stock cultures of each strain were streaked onto tryptic soy agar (TSA; Difco, Becton Dickinson & Co., Sparks, MD) with 80 µg/ml rifampicin (TSAR; *E. coli* O157:H7 and *Salmonella*) or 50 µg/ml nalidixic acid (TSAN; *L. monocytogenes*) and incubated at 35 ± 2 °C for 24 h. A single colony from each strain was then transferred to 10 ml tryptic soy broth (TSB; Bacto, Becton, Dickinson & Co., Sparks, MD) with 80 µg/ml rifampicin (TSBR; *E. coli* O157:H7 and *Salmonella*) or 50 µg/ml nalidixic acid (TSBN; *L. monocytogenes*) two times at 24 h intervals and incubated at 35 ± 2 °C. Immediately prior to use, each culture was centrifuged at $3000 \times g$ for 10 min (Allegra X-12, Beckman Coulter, Fullerton, CA). Cells were washed twice in 10 ml, 0.1% peptone (Bacto, Becton, Dickinson & Co., Sparks, MD). Washed cells were suspended in half the original culture volume of 0.1% peptone. Each strain was enumerated following serial dilutions in 0.1% peptone, on TSAR (*E. coli* O157:H7 and *Salmonella*) or TSAN (*L. monocytogenes*) to confirm cell concentration. All strains were combined in equal

volumes (2 ml of each strain) to prepare the cocktail. The cocktail was diluted in 0.1% peptone to arrive at a final inoculum concentration of ca. 10^7 CFU/ml. Cocktails were stored on ice for up to 1 h prior to sample inoculation.

2.4. Inoculation and storage

Celery samples were spot-inoculated with 10 µl (3–5 drops) of cocktail onto cut or exterior uncut surfaces, prior to drying at ambient temperature for 1 h in a biosafety cabinet. After drying, each 10-g sample was individually transferred to, and sealed within, a 16.5×8.25 cm polyethylene plastic press-to-seal snack bag (Great Value®, Walmart Stores, Inc., Bentonville, AR) or a 414 ml polyethylene “double-seal lidded” container (Gladware® Side Dish, Glad Manufacturing Co., Amherst, VA) and stored 4 ± 2 °C, 12 ± 2 °C, or 22 ± 2 °C. Press and seal bags, and lidded double seal containers were selected to mimic containers that may be used by consumers to store cut celery.

2.5. Enumeration of pathogens

Bacterial populations were enumerated from celery samples at different storage temperatures on the following days: as follows: Samples incubated at 4 or 12 ± 2 °C, were enumerated on days 0, 1, 3, 5, and 7; samples incubated at 22 ± 2 °C, were enumerated at 0, 8, and 17 h, and 1, and 2 days. At each sampling point, samples were transferred into 1.6 l sterile stomacher bags (Whirl-Pak, Nasco, Modesto, CA), combined with 40 ml of 0.1% peptone, and macerated in a stomacher (Smasher, AES Lab, Cranbury, NJ) at high speed for 1 min. Serial dilutions of each sample were made in 0.1% peptone and surface plated (0.1 ml) in duplicate onto selective and nonselective media containing rifampicin or nalidixic acid. To increase the limit of detection to 0.6 log CFU/g, 1 ml of the lowest dilution was distributed evenly over four plates (0.25 ml/plate).

E. coli O157:H7 populations were enumerated using TSAR as a nonselective media, and sorbitol MacConkey agar (SMAC; Difco, Becton Dickinson & Co.) supplemented with rifampicin (SMACR) as a selective media. Nalidixic acid did not sufficiently reduce background populations on celery, thus *L. monocytogenes* populations were only enumerated on selective modified Oxford agar supplemented nalidixic acid (MOXN; Difco, Becton Dickinson & Co.). *Salmonella* populations were enumerated using TSAR as a nonselective media, and bismuth sulfite agar supplemented with rifampicin (BSAR; Difco, Becton Dickinson & Co.) as a selective media. Uninoculated samples were plated onto nonselective and selective media, as well as onto TSA to determine background microflora. These samples were incubated at 35 ± 2 °C, for 24 h (nonselective media) or 48 h (selective). Colonies were counted by hand and populations were expressed as log CFU/g celery.

2.6. Atmosphere modification during storage

Uninoculated celery in a sample bag or container was held at 4, 12, or 22 °C for up to 7 days and atmospheric probes (CheckMate 9900, O₂/CO₂ analyzer; PBI Dansensor, Glen Rock, NJ) recorded data on O₂ and CO₂ levels once every 24 h.

2.7. Statistical analyses

Data were statistically evaluated via multiple Tukey's-adjusted ANOVAs using SAS 9.2 for Windows (SAS Manufacturing, Inc., 405 N. Smith Ave, Corona, CA 92880). All experimental factors were compared simultaneously for each pathogen. Comparisons of mean log CFU/g were made between bagged and container-stored samples, cut and uncut inoculation surfaces, media types

(nonselective and selective), and between as well as within time intervals. Possible combined effects between storage container type and site of surface inoculation were also examined. Comparisons were also made between temperatures, but not between pathogens. Differences were considered significant at $P \leq 0.05$. Growth rates were plotted using Microsoft Excel 2010, and DMfit v2.1 was used to model growth rates (Baranyi and Roberts, 1994).

3. Results

No *E. coli* O157:H7, *L. monocytogenes* or *Salmonella* was observed in any of the control samples. No significant differences in growth were observed between any of the strains during cocktail preparation ($P \geq 0.05$). Population decreases of up to 1 log CFU/g from initial inoculation levels were observed during air drying. The average log CFU/g populations of *E. coli* O157:H7 and *Salmonella* enumerated on nonselective (TSAR) and selective (SMACR for *E. coli* O157:H7 and BSAR for *Salmonella*) media on each sampling day were not significantly different ($P \geq 0.05$); TSAN results are discussed below; growth rates on both selective and nonselective media are presented in Table 1. A relatively low passive accumulation of CO₂ was measured in the sealed bags of celery ($\leq 2.4\%$, data not shown), and significant differences were not seen between container types.

3.1. Background microflora on fresh-cut celery

Background, total aerobic plate counts ranged from ca. 6 to 10 log CFU/g celery (data not shown). Among samples, background levels were highly variable and diverse populations were noted (i.e., visually noticeable differences in color and colony morphology). No consistent increases or decreases in total aerobic counts were observed during storage at any temperature (data not shown).

3.2. Fate of *E. coli* O157:H7 on celery

At 4 ± 2 °C over 7 days, populations of *E. coli* O157:H7 decreased significantly ($P \leq 0.05$) under all storage conditions by 1–2 log CFU/g (Fig. 1A); growth rates (from nonselective media) ranged from -0.34 to -0.21 log CFU/g/day (Table 1). Initial (0 h) mean *E. coli* O157:H7 population size was significantly lower on cut versus uncut inoculation surfaces ($P \leq 0.05$). Populations of *E. coli* O157:H7 on celery did not change significantly between days 0 and 1, or 3 and 5 ($P \geq 0.05$), while significant decreases, under all conditions ($P \leq 0.05$), were noted between days 1 and 3, and 5 and 7. Populations on cut surfaces and stored in containers were significantly smaller than those on uncut surfaces and stored in containers, and samples stored in bags regardless of surface type ($P \leq 0.05$), and the largest decreases were observed on samples stored in containers and inoculated on uncut surfaces (Fig. 1A; open squares).

At 12 ± 2 °C, *E. coli* O157:H7 populations increased slightly (less than 1 log CFU), but statistically significantly ($P \leq 0.05$), from day 0 to day 1 and then decreased significantly between days 1 and 3 ($P \leq 0.05$), resulting in no significant change in population size over 7 days ($P \geq 0.05$; Fig. 1B). No significant population size differences were observed between container types or inoculation site ($P \geq 0.05$). Growth rates of *E. coli* O157:H7 (from nonselective media) at 12 ± 2 °C ranged from -0.11 to -0.04 log CFU/g/day (Table 1).

On fresh-cut celery held at 22 ± 2 °C, *E. coli* O157:H7 populations increased by ca. 1.0 log CFU/g, with the majority of growth occurring over the first 17 h (Fig. 1C). Between 0 and 8 h, the *E. coli* O157:H7 population size did not change significantly ($P \geq 0.05$), but increased significantly between 8 and 17 h ($P \leq 0.05$). *E. coli* O157:H7 populations inoculated on cut celery surfaces were significantly larger than those on uncut surfaces ($P \leq 0.05$) during storage. No significant population size differences were observed between container types ($P \geq 0.05$). Growth rates of *E. coli* O157:H7

Table 1

Pathogen growth rate (μ ; expressed as log CFU/g/day) with positive growth rates shown in bold and correlation coefficient (r^2 -value) for each treatment combination, as determined by DMfit ($n = 6$).

Temperature	Treatment ^a			<i>E. coli</i> O157:H7		<i>L. monocytogenes</i>		<i>Salmonella</i>	
	Bag/Container	Cut/Uncut	Selective/Nonselective	μ	r^2	μ^b	r^2	μ	r^2
4 °C	Bag	Cut	Nonselective	-0.21	0.83			-0.13	0.73
			Selective	-0.29	0.88	-0.18	0.69	-0.15	0.90
	Uncut	Nonselective	-0.22	0.59			-0.14	0.65	
		Selective	-0.20	0.46	-0.16	0.97	-0.11	0.92	
	Container	Cut	Nonselective	-0.33	0.88			-0.11	0.85
			Selective	-0.65	1.0	-0.21	0.71	-0.15	0.83
12 °C	Bag	Cut	Nonselective	-0.34	0.75			-0.19	0.74
			Selective	-0.28	0.56	-0.22	0.55	-0.13	0.65
	Uncut	Nonselective	-0.07	0.25			-0.06	0.63	
		Selective	-0.06	0.13	0.07	0.26	-0.09	0.79	
	Container	Cut	Nonselective	-0.04	0.00			-0.02	0.76
			Selective	0.04	0.00	0.13	0.18	0.00	0.00
22 °C	Bag	Cut	Nonselective	-0.11	0.64			-0.05	0.59
			Selective	-0.10	0.52	0.14	0.40	-0.07	0.71
	Uncut	Nonselective	-0.04	0.20			-0.04	0.12	
		Selective	0.05	0.00	0.15	0.64	-0.03	0.00	
	Container	Cut	Nonselective	0.66^c	0.93			3.9	0.99
			Selective	0.49^c	0.82	0.39	0.40	3.6	0.94
Uncut	Nonselective	0.54^c	0.56			1.8^c	0.81		
	Selective	1.6	0.92	0.34	0.01	1.7^c	0.81		
Container	Cut	Nonselective	0.54^c	0.99			3.2^c	0.81	
		Selective	0.39^c	0.96	0.46	0.40	0.9	1.00	
Uncut	Nonselective	0.41^c	0.48			1.3	0.99		
	Selective	1.3^c	1.00	0.37	0.19	1.5^c	0.94		

^a Identifies storage condition (bag or container), inoculation surface (cut or uncut), and recovery media (selective or nonselective).

^b Due to background populations, *L. monocytogenes* could not be recovered from nonselective media.

^c In some cases, DMfit was unable to fit an accurate trend line to the growth curves, here a regression line was fit in Excel between three data points of the growth curve.

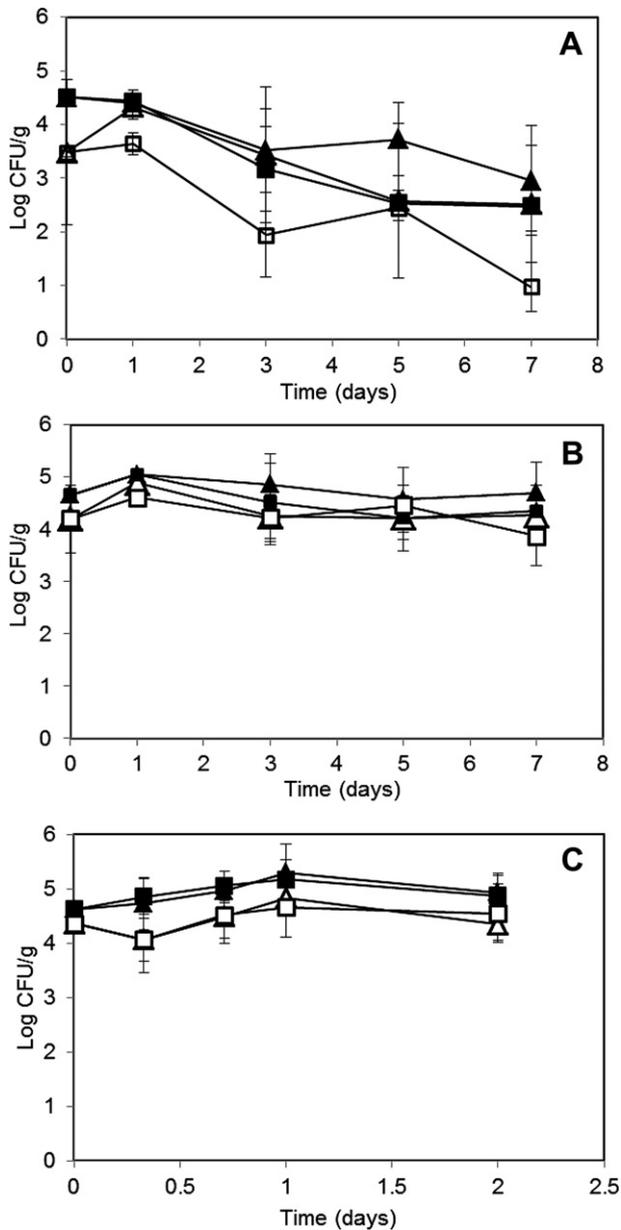


Fig. 1. Behavior of *E. coli* O157:H7 inoculated onto cut (solid) or uncut (open) celery surfaces and stored in bags (\blacktriangle) or container (\blacksquare) held at 4 ± 2 °C (A) and 12 ± 2 °C (B) for 7 days and at 22 ± 2 °C (C) for 2 days. Populations determined on TSAR; values are the means of replicate samples ($n = 6$); error bars represent standard deviations.

(from nonselective media) at 22 ± 2 °C ranged from 0.41 to 0.66 log CFU/g/day (Table 1).

3.3. Fate of *L. monocytogenes* on celery

At 4 ± 2 °C, populations of *L. monocytogenes* decreased significantly under all storage conditions ($P \leq 0.05$) by ca. 1 log CFU/g over 7 days (Fig. 2A); growth rates ranged from -0.22 to -0.16 log CFU/g/day (Table 1). The largest decreases were observed on samples stored in containers and inoculated on uncut surfaces (open squares). Populations inoculated on cut surfaces and stored in containers were significantly smaller than under all other conditions ($P \leq 0.05$).

Small (0.5 log CFU/g) but significant ($P \leq 0.05$) increases in *L. monocytogenes* population size, were noted over 7 days, with

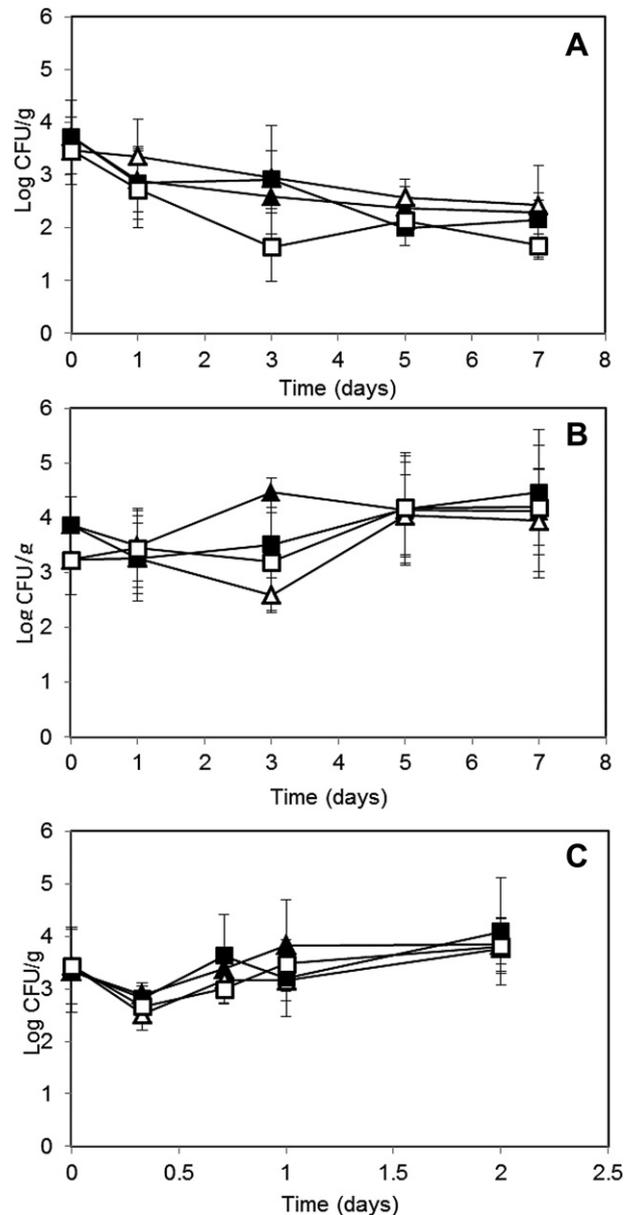


Fig. 2. Behavior of *L. monocytogenes* inoculated onto cut (solid) or uncut (open) celery surfaces and stored in bags (\blacktriangle) or container (\blacksquare) held at 4 ± 2 °C (A) and 12 ± 2 °C (B) for 7 days and at 22 ± 2 °C (C) for 2 days. Populations determined on MOXN; values are the means of replicate samples ($n = 6$); error bars represent standard deviations.

L. monocytogenes increasing significantly from day 0 to day 7 by ca. 0.5 log CFU/g ($P \leq 0.05$) at 12 ± 2 °C (Fig. 2B). Growth rates of *L. monocytogenes* ranged from 0.07 to 0.15 log CFU/g/day (Table 1). Increases occurred primarily between days 3 and 5, which were significant under all conditions ($P \leq 0.05$), with the exception of samples inoculated on cut surfaces and stored in bags ($P \geq 0.05$). No significant population size differences were observed between container types ($P \geq 0.05$).

On fresh-cut celery held at 22 ± 2 °C, *L. monocytogenes* populations increased by ca. 0.5 log CFU/g in 2 days (Fig. 2C). *L. monocytogenes* population initially decreased significantly from 0 to 8 h by ca. 0.7 log CFU/g, but overall increases between day 0 and day 2 were significant ($P = 0.04$). This increase was most apparent between 8 and 17 h, where populations increased significantly by ca. 1 log CFU/g ($P \leq 0.05$). No significant population size differences were observed between container types or inoculation site

($P \geq 0.05$). Growth rates of *L. monocytogenes* at $22 \pm 2^\circ\text{C}$ ranged from 0.34 to 0.46 log CFU/g/day (Table 1).

3.4. Fate of *Salmonella* spp. on celery

At $4 \pm 2^\circ\text{C}$, over 7 days, populations of *Salmonella* decreased significantly under all storage conditions ($P \leq 0.05$) by 0.5–1 log CFU/g (Fig. 3A); growth rates (on nonselective media) ranged from -0.19 to -0.11 log CFU/g/day (Table 1). The largest decreases were observed on samples stored in containers and inoculated on uncut surfaces (open squares). No significant differences in *Salmonella* population survival were seen between container type ($P \geq 0.05$). However, mean populations were significantly greater on cut surfaces compared to uncut sites throughout the experiment ($P \leq 0.05$), with the exception of day 0 ($P \geq 0.05$; Fig. 3A).

At $12 \pm 2^\circ\text{C}$, *Salmonella* populations did not change significantly between any of the sampling dates ($P \geq 0.05$; Fig. 3B) and growth rates (from nonselective media) ranged from -0.02 to -0.06 log CFU/g/day (Table 1). Initial (0 h) inoculum levels were significantly larger on samples inoculated on cut surfaces ($P \leq 0.05$). No significant population differences were observed between container types ($P \geq 0.05$).

On fresh-cut celery held at $22 \pm 2^\circ\text{C}$, *Salmonella* populations increased by ca. 2.0 log CFU/g (Fig. 3C), with the majority of growth occurring over the first 0.71 day at growth rates (nonselective media) ranging between 1.3 and 3.9 log CFU/g/day (Table 1). With the exception of day 0, *Salmonella* populations inoculated on cut celery surfaces were significantly larger than those on uncut surfaces ($P \leq 0.05$). Significant difference in *Salmonella* concentrations between container types was observed at $22 \pm 2^\circ\text{C}$ on day 2, when populations on cut surfaces stored in containers were larger than those stored in bags ($P \leq 0.05$).

4. Discussion

High levels of organisms naturally occurring on celery were not unexpected; total aerobic counts on 26, 100 g raw celery samples ranged from 2 log CFU/g up to 8.3 log CFU/g (Garcia-Villanova et al., 1987) and fluctuations in the direction of population change during storage is also consistent with previously documented storage studies at 4 and 10°C for up to 11 days (Odumeru et al., 1997). The large and diverse background populations inherent to celery may have contributed to the behavior of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella*, with *L. monocytogenes* being particularly sensitive to competitive microflora, including lactic acid bacteria and enterobacteria (Francis and O'Beirne, 1998a; Francis and O'Beirne, 1998b; Vescovo et al., 1996). Large endogenous populations and the relatively limited availability of nutrients in celery (US FDA, 2009), including differences in nutrient availability and water activity between cut and uncut surfaces (Castro-Rosas et al., 2010), may have contributed to the relatively underwhelming growth of pathogens, even at elevated temperatures seen in experimental results. No association with the initial background levels and the pathogen response was noted.

The highest growth rates of a foodborne pathogen tested here were those of *Salmonella* on the cut surface of celery stored at 22°C estimated at ca. 4 log CFU/g increase per day; populations levels of *Salmonella* and *E. coli* O157:H7 did not exceed ca. 5.5 CFU/g even under these conditions of maximum growth, and ca. 2 log CFU/g increases over 2 days. A flaw of the growth rate models developed here is that they do not take into account what the maximum population load may be on fresh-cut celery, and a portion of the predicted 4 log increase of *Salmonella* is not realized. Similar maximum population densities have been noted for *Salmonella* on

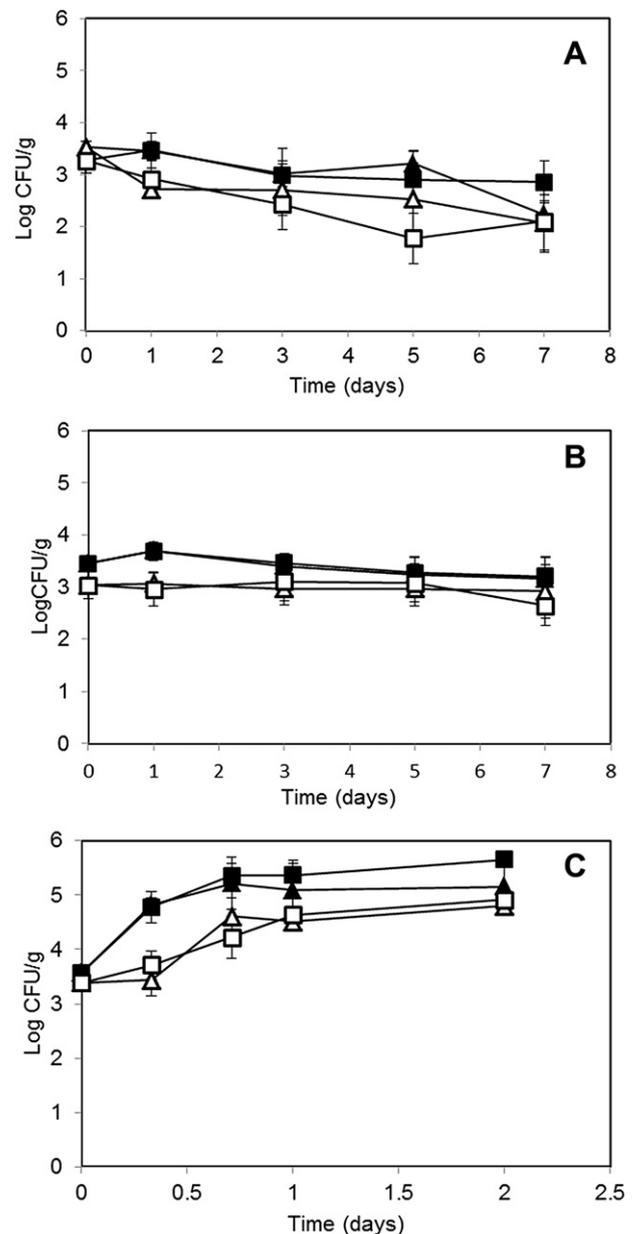


Fig. 3. Behavior of *Salmonella* (C) inoculated onto cut (solid) or uncut (open) celery surfaces and stored in bags (\blacktriangle) or container (\blacksquare) held at $4 \pm 2^\circ\text{C}$ (A) and $12 \pm 2^\circ\text{C}$ (B) for 7 days and at $22 \pm 2^\circ\text{C}$ (C) for 2 days. Populations determined on TSAR; values are the means of replicate samples ($n = 6$); error bars represent standard deviations.

tomatoes stored at 30°C (Zhuang et al., 1995), and for *Salmonella* and *E. coli* O157:H7 on papaya stored at 23°C (Strawn and Danyluk, 2010b), while 8 log CFU/g of *Salmonella* and *E. coli* O157:H7 could be recovered from minimally processed peaches held at 25°C (Alegre et al., 2010). *Salmonella* populations on fresh Italian parsley increased to 4.1 log CFU/g over 7 days at 25°C (Duffy et al., 2005) similar to what we have noted here on celery. Conversely, *Salmonella* populations inoculated onto alfalfa sprouts decreased at 25°C (Gandhi et al., 2001; Taormina and Beuchat, 1999); the waxy cuticle of the sprout repelling the inoculum mixture was cited as a possible explanation. These observations coupled with those on fresh-cut celery make it apparent that pathogen behavior is often unique to the produce item in question.

The behavior of foodborne pathogens at refrigeration temperatures on a variety of fresh and fresh-cut produce is well

established (Aruscavage et al., 2006; Heaton and Jones, 2008). Comparable behavior to the slow *Salmonella* and *E. coli* O157:H7 population declines at 4 °C, over multiple days, shown here has been reported on minimally processed alfalfa sprouts, diced celery, leafy greens and herbs, rutabaga, soybean sprouts, papaya, peaches, pineapple, and zucchini squash (Alegre et al., 2010; Castro-Rosas et al., 2010; Duffy et al., 2005; Francis and O'Beirne, 2001; Gandhi et al., 2001; Hsu et al., 2006; Kakiomenou et al., 1998; Prakash et al., 2000; Strawn and Danyluk, 2010a,b; Weissinger et al., 2000). Ravishankar et al. (2010), report a similarly slow decline (0.3–0.5 log CFU/g) in *Salmonella* Newport populations on 10 g cut celery samples, dip inoculated and stored in a sterile Petri dish at 4 °C for 3 days. Similarly, *L. monocytogenes* did not grow at 4 °C on rutabaga or shredded lettuce, and declined on lettuce, dry coleslaw, shredded carrots, soybean sprouts, respectively (Francis and O'Beirne, 2001; Kakiomenou et al., 1998). In contrast to the behavior of *L. monocytogenes* on the products described above at 4 °C, *Listeria innocua* populations inoculated onto peaches held at 5 °C increased by ca. 0.5 log CFU/g over 6 days (Alegre et al., 2010).

Less is known about the fate of foodborne pathogens on produce held at elevated temperatures that are above recommended refrigeration but below normal room temperatures. With the exception of a slight increase in *L. monocytogenes* populations over 7 days, populations generally remain stable on both cut and uncut surfaces of celery at 12 °C, similar to what has been observed for *E. coli* O157:H7 and *L. monocytogenes* on soybean sprouts over 5 days at 8 °C (Francis and O'Beirne, 2001) and *E. coli* O157:H7 populations on mangos over 10 days at 12 °C (Strawn and Danyluk, 2010b). Nutrient availability, pH, or other intrinsic factors of fresh-cut items likely play a major role in pathogen proliferation at abusive temperatures. *Salmonella* and *E. coli* O157:H7 populations increased on fresh-cut papaya over 10 days at 12 °C (Strawn and Danyluk, 2010b); *Salmonella* increased on cut mango over 10 days at 12 °C (Strawn and Danyluk, 2010b); *L. monocytogenes* and *E. coli* O157:H7 increased on shredded lettuce and rutabaga over 5 days at 8 °C (Francis and O'Beirne, 2001); and *L. innocua* increased on fresh-cut peaches over 6 days at 10 °C (Alegre et al., 2010). Conversely, *Salmonella* and *E. coli* O157:H7 populations slowly decreased on cut pineapple stored at 12 °C (Strawn and Danyluk, 2010a), and *L. monocytogenes* and *E. coli* O157:H7 slowly decreased on packaged coleslaw at 8 °C (Francis and O'Beirne, 2001).

In general, pathogens inoculated onto the freshly cut surfaces of celery showed greater multiplication than those on uncut surfaces, similar to our observations of inocula location-based behavioral differences, at 25 °C, *Salmonella* and *E. coli* O157:H7 populations inoculated onto the skin vs. flesh of zucchini squash at 25 °C: *Salmonella* and *E. coli* O157:H7 populations decreased over 7 days on the skin of whole zucchini squash, while those inoculated onto the flesh of zucchini slices increased over the same period (Castro-Rosas et al., 2010). Similarly, investigators found that *E. coli* O157:H7 populations survived longer on mechanically damaged lettuce leaves compared to undamaged leaves (Aruscavage et al., 2008).

Survival of a pathogenic microorganism on produce is dictated by its metabolic capabilities, which can be greatly influenced by intrinsic and extrinsic environmental factors (Beuchat, 2002). Modified atmospheres can naturally develop within sealed containers of fresh produce due to plant cell respiration, and the concentrations of O₂ and CO₂ within packages can vary by food product (Francis and O'Beirne, 2001). Atmospheric conditions of up to 30% CO₂ do not inhibit the growth of *E. coli* O157:H7 on vegetables (Diaz and Hotchkiss, 1996; Francis and O'Beirne, 2001), while *L. monocytogenes* is known to be sensitive to certain atmospheric changes, including high CO₂ concentrations (Francis and O'Beirne, 2001). However, the relatively modest atmospheric changes observed in packages containing lettuce and rutabaga (similar to

those observed here) were not found to affect the fate of *L. monocytogenes*, while the atmospheric changes in packages containing soybean sprouts and coleslaw mix did suppress *L. monocytogenes* growth (Francis and O'Beirne, 2001). The inhibitory effect of high CO₂ concentrations on *L. monocytogenes* is related to decreased pH and interference with cell metabolism (Dixon and Kell, 1989). Headspace atmosphere, sample pH, and background microflora have been attributed to the lack of *L. monocytogenes* growth on shredded carrots and lettuce (Kakiomenou et al., 1998). Considering the low passive accumulation of CO₂ by packaged celery and reported pH values of 5.7–6.0 (US FDA, 2007), the passive modified atmospheres and pH are unlikely to have inhibited pathogen growth in our study.

The data presented here describe the potential in fresh-cut celery for *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* survival and growth at a variety of temperatures, and minimal impact of simulated consumer packaging container on pathogen fate. Current consumer recommendations to maintain cut celery below 4 °C to prevent the growth of pathogens are consistent with the results presented here. The growth rates predicted here can be used in subsequent quantitative microbial risk assessments for fresh-cut celery to estimate the effect of storage temperature on pathogen populations, however caution should be used such that maximum concentration densities do not overestimate population size.

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