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Survival or Growth of Inoculated *Escherichia coli* O157:H7 and *Salmonella* on Yellow Onions (*Allium cepa*) under Conditions Simulating Food Service and Consumer Handling and Storage

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ABSTRACT

Whole and diced yellow onions (*Allium cepa*) were inoculated with five-strain cocktails of rifampin-resistant *Escherichia coli* O157:H7 or *Salmonella* and stored under conditions to simulate food service or consumer handling. The inoculum was grown in broth (for both whole and diced onion experiments) or on agar plates (for whole onion experiments). Marked circles (3.3 cm in diameter) on the outer papery skin of whole onions were spot inoculated (10 μl in 10 drops) at 7 log CFU per circle, and onions were stored at 4°C, 30 to 50% relative humidity, or at ambient conditions (23°C, 30 to 50% relative humidity). Diced onions were inoculated at 3 log CFU/g and then stored in open or closed containers at 4°C or ambient conditions. Previously inoculated and ambient-stored diced onions were also mixed 1:9 (wt/wt) with refrigerated uninoculated freshly diced onions and stored in closed containers at ambient conditions. Inoculated pathogens were recovered in 0.1% peptone and plated onto selective and nonselective media supplemented with 50 μg/ml rifampin. Both *E. coli* O157:H7 and *Salmonella* populations declined more rapidly on onion skins when the inoculum was prepared in broth rather than on agar. Agar-prepared *E. coli* O157:H7 and *Salmonella* declined by 0.4 and 0.3 log CFU per sample per day, respectively, at ambient conditions; at 4°C the rates of reduction were 0.08 and 0.06 log CFU per sample per day for *E. coli* O157:H7 and *Salmonella,* respectively. Populations of *E. coli* O157:H7 and *Salmonella* did not change over 6 days of storage at 4°C in diced onions. Lag times of 6 to 9 h were observed with freshly inoculated onion at ambient conditions; no lag was observed when previously inoculated and uninoculated onions were mixed. Growth rates at ambient conditions were 0.2 to 0.3 log CFU/g/h for *E. coli* O157:H7 and *Salmonella* in freshly inoculated onion and 0.2 log CFU/g/h in mixed product. Diced onions support pathogen growth and should be kept refrigerated.

The ''Spanish onion,'' also known as the yellow, brown, red, or white bulb onion (*Allium cepa*), is widely grown around the world *(3).* Onions are typically harvested in the United States by mechanically undercutting about 2 to 5 cm below the bottoms of the bulbs. In arid regions, undercut onions can be cured (dried) in the field for 2 to 4 weeks; in wetter climates, onions may be harvested immediately after undercutting and then cured indoors using forced heated air *(23).* Whole onions that are properly dried have a shelf life of 1 to 9 months when stored close to 0°C and below 65 to 70% relative humidity (RH) *(23).* *Escherichia coli* O157:H7 present in contaminated irrigation water or manure persists for several weeks and can transfer to the onion bulb *(12),* but survival of the organism during curing has not been evaluated.

Onions may be consumed as an ingredient in a wide array of cooked savory dishes or raw as a component or garnish on salads or as a condiment on meats. A variety of commercially prepared fresh-cut onions are available for food service (peeled, sliced, slivered, ring, diced, and pureed) and retail (diced) markets. The shelf life of fresh-cut onions is approximately 14 days, with proper storage and packaging (4°C and modified atmosphere of 2% O_2 and 10% CO_2) *(2).*

Bulb onions have rarely been associated with recalls or outbreaks of foodborne illness. Fresh-cut bulb onions were recalled in 2012 after isolation of *Listeria monocytogenes,* but no cases of listeriosis were reported *(26).* In 1983, 28 people fell ill with type A botulism from consuming patty melts, a type of sandwich *(18),* and *Clostridium botulinum* was isolated from some of the raw onions remaining in the restaurant. Raw onions had been sautéed in margarine and then held for long periods at the back of the grill before being used to garnish the sandwiches. The authors hypothesized that these handling procedures provided a warm, high water activity, anaerobic environment suitable for *C. botulinum* to grow and produce toxin.

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A fast food chain restaurant in North Bay, Ontario, Canada *(21),* was implicated in a month-long 2008 outbreak of 235 reported cases of *E. coli* O157:H7 gastroenteritis. A case-control analysis identified the most likely cause of the outbreak as uncooked diced onions that were used as a garnish on hamburgers. The onion garnish was prepared in the restaurant by hand peeling whole onions (on a cutting board with a knife and use of a protective cutting glove) and then chopping using a metal dicer directly into a plastic storage container. Although not confirmed as the source, the investigators concluded that inconsistent and insufficient cleaning of the onion dicer may have contributed to contamination of the diced onions as they were prepared. The chopped onions were stored in bulk containers in the refrigerator and periodically transferred to small bowls at the garnish stations. The onions at the garnish stations were held at room temperature, and the bowls were topped up during the day.

L. monocytogenes survived but did not grow in modified atmosphere–packaged sliced onion held at 4°C and increased by approximately 1 log CFU/g over 6 days at 10°C *(9)*. Little is known about the survival or growth of other foodborne pathogens on onion skin or on cut onions. The objectives of the current study were to evaluate whether *E. coli* O157:H7 and *Salmonella* survive on the skins of whole onions and in diced onions during refrigerated and ambient storage and under conditions that simulate consumer or food service handling.

MATERIALS AND METHODS

Onion samples. Whole yellow onions with smooth outer skins, selected from bulk bins, and commercial fresh-cut (diced) yellow onions were purchased at local retail markets (Davis, CA). The whole onions were stored at ambient conditions (23 ± 0.3°C, 30 to 50% RH, median 35% RH), and the fresh-cut onions were stored in original packaging (either a sealed plastic bag or sealed plastic container) at 4°C for up to 1 day before use.

Bacterial cultures. The strains used in this study were the same as those used previously with fresh-cut celery *(28)*: *Salmonella enterica* Montevideo (G4639), a clinical isolate from an outbreak associated with tomatoes *(10)* (provided by Dr. Larry R. Beuchat [LRB], University of Georgia, Griffin); *Salmonella enterica* Enteritidis PT 30 (ATCC BAA-1045), isolated from raw almonds associated with a 2000 to 2001 outbreak *(11)*; *Salmonella enterica* Agona isolated from alfalfa sprouts (LRB); *Salmonella enterica* Michigan, a clinical isolate from an outbreak associated with cantaloupe (LRB); *Salmonella enterica* Gaminara (F2712), isolated from orange juice associated with the 1995 outbreak *(7)* (LRB); *E. coli* O157:H7 (Odwalla strain 223), a clinical isolate from an outbreak associated with apple juice *(5)*; *E. coli* O157:H7 (CDC658), a clinical isolate from an outbreak associated with cantaloupe *(8)* (LRB); *E. coli* O157:H7 (F4546), a clinical isolate from an outbreak associated with alfalfa sprouts *(4)* (LRB); *E. coli* O157:H7 (EC4042), a clinical isolate from an outbreak associated with spinach *(15)*; and *E. coli* O157:H7 (H1730), a clinical isolate from an outbreak associated with lettuce *(1)* (LRB). All cultures were stored at −80°C in tryptic soy broth (TSB) supplemented with 15% glycerol.

Preparation of inocula. Individual frozen stock cultures were streaked onto tryptic soy agar (TSA) and incubated at 37°C overnight. Isolated colonies were transferred into 10 ml of TSB supplemented with rifampin (R) at 50 μg/ml (TSBR), and then incubated at 37°C overnight. For inocula collected from agar plates, individual strains were first transferred into TSB, as described previously, and then each overnight culture (1 ml) was plated onto TSA supplemented with 50 μg/ml of R (TSAR), and plates were incubated at 37°C overnight. After incubation, 0.1% peptone (9 ml) was added to each plate, and the cell lawn was collected with an L-shaped plate spreader. Five-strain pathogen cocktails were prepared by combining 5 ml of each culture. For broth-prepared inoculum, the second overnight culture (1 ml) was placed into a sterile centrifuge tube (1.5 ml) and centrifuged at 16,000 × *g* for 2 min. The supernatant was discarded, and the pellet was washed twice in 1 ml of 0.1% peptone. After washing, each cell pellet was suspended in 1 ml of sterile Milli-Q water. Separate five-strain cocktails were prepared for *E. coli* O157:H7 and *Salmonella* by combining equal volumes (200 μl) of cell suspensions from each strain. Dilutions were prepared in sterile Milli-Q water to achieve initial target populations. Unless otherwise specified, all media were from Difco, BD (Franklin Lakes, NJ).

Preparation and inoculation of onions. Four to five circles (3.3 cm in diameter) were drawn on the outermost papery skin of whole onions around the circumference to define the inoculation area. The prepared inoculum (10 μl) of either *E. coli* O157:H7 or *Salmonella* was distributed in 10, 1-μl spots over each marked area to give an initial inoculum of 7 log CFU per circle. Inoculated whole onions were held in a biosafety cabinet for 30 min to allow the inoculum to dry; onions were then transferred to the appropriate storage condition. At the time of sampling, the inoculated areas were excised from the whole onions by cutting and removing the outermost papery skin layer. Each excised circle of papery skin corresponded to approximately 1 g of onion skin.

To prepare diced onions, uninoculated whole yellow onions were peeled (papery skin removed) and cut into quarters with a knife, the quarters were placed in a manual vegetable chopper (Vidalia Chop Wizard, National Express Online, Norwalk, CT), and then diced into pieces (approximately 1 by 1 cm). Diced onions (20 g) were weighed into a 240-ml specimen storage container (Thermo Fisher Scientific, Waltham, MA) and 20 μl of either the *E. coli* O157:H7 or *Salmonella* cocktail was added to give a target inoculum of 4 log CFU/g. After inoculation, diced onions were mixed by gently shaking the storage containers at a 30° angle for 25 s. Control samples were mixed with 20 μl of sterile Milli-Q water.

Handling and storage conditions. Inoculated whole onions were stored at 4 ± 0.6°C (30 to 50% RH) or at ambient conditions for up to 56 days. Microbial levels on the onion skin were determined on samples excised immediately after inoculation, after 30 min of drying (day 0 of storage), on day 1, and every 7 or 14 days up to 56 days.

Diced onions in specimen containers were stored as follows: (i) commercial fresh-cut and freshly diced onions in closed containers were held at ambient conditions (23 ± 0.3°C, 30 to 50% RH, median 35% RH); (ii) freshly diced onions in open or closed containers were held at ambient conditions; and (iii) freshly diced onions in closed containers were held at 4 ± 0.6°C. *E. coli* O157:H7 and *Salmonella* levels were determined for all samples immediately after inoculation: for samples (i) at 8, 10, 12, 14, 16, 20, 24, and 36 h after inoculation; for samples (ii) at 12 and 24 h; and for samples (iii) at 8 h and days 1, 4, and 6 of storage. Levels of background mesophilic microbiota were also determined at the same times for uninoculated control samples.

The practice of topping off ambient-stored diced onions with refrigerated freshly diced onions was simulated in the following way: inoculated freshly diced onions were stored at ambient conditions for 12 h in closed containers and then combined 1:9 (wt/wt) with refrigerated uninoculated freshly diced onions and stored for an additional 30 h under the same ambient conditions. Microbial levels were determined at the intervals described for storage condition (i) previously. Fresh and 12-h mixed product was sampled immediately after mixing and every 2 h through 12 h and at 24 and 30 h after inoculation.

Samples were held on a laboratory bench in large plastic bins to simulate storage at ambient conditions. Samples were stored at 4°C in a Revco refrigerated incubator (Thermo Fisher Scientific). Temperature and relative humidity were recorded using a data logger (TempTale 4, Sensitech Inc., Beverly, MA).

Bacterial recovery and enumeration. Onion skins were sampled by aseptically excising the outermost papery skin layer at the marked circles with a sterile scalpel. Each excised skin sample was placed into 3 ml of 0.1% peptone in a 120-ml Whirl-Pak bag (Nasco, Modesto, CA), and samples were shaken by hand for 30 s, rubbed for 15 s, and shaken again for an additional 30 s. The entire diced onion sample (20 g) was transferred into a 200-ml Whirl-Pak filter bag containing 40 ml of 0.1% peptone. Samples were homogenized (Stomacher 400 Circulator, Seward, Bohemia, NY) for 2 min at the high setting.

Serial dilutions of the prepared samples were made in 0.1% peptone. Dilutions were plated in duplicate onto media supplemented with R at 50 μg/ml, including TSAR, *E. coli* O157:H7–selective sorbitol MacConkey agar (SMACR), or *Salmonella* selective bismuth sulfite agar (BSAR). Uninoculated control samples were plated onto TSA to determine levels of background microbiota.

When counts on the onion skin were expected to be near or below the limit of detection, either the entire sample or material remaining after plating (approximately 1 ml) was enriched by adding 10 ml of TSB to each sample and then incubating at 37°C overnight and streaking onto SMACR or BSAR. Samples were incubated at 37°C for 24 ± 4 h (TSA and TSAR) or 48 ± 4 h (BSAR and SMACR).

Statistical analysis. Three inoculated and two uninoculated replicates of onion skin circles from three different onions were excised at each sampling point. Samples of diced onions were weighed into separate containers (20 g per container), and each container was inoculated individually. Three replicates were analyzed at each sampling point. Each experiment was replicated two or three times for a total of six or nine analytical units at each time point; the mean of all replicates was reported, and the standard deviation was calculated for each value. Some preliminary data collection experiments were not replicated.

Analysis of variance and the post hoc Tukey's honest significant difference test were calculated by using JMP 10 software (SAS Institute, Cary, NC). The mean log CFU per gram was compared between commercial diced and freshly diced onions, onions stored in open and closed containers, and mixed and unmixed samples. Variances in the mean values were considered statistically significant at $P < 0.05$. *E. coli* O157:H7 or *Salmonella* growth curves on freshly diced onions at ambient conditions were analyzed with the DMFit (www.combase.cc) add-in for Excel (Microsoft, Redmond, WA).

RESULTS AND DISCUSSION

Survival of *E. coli* O157:H7 and *Salmonella* on onion skins. In preliminary experiments the potential for transfer of the inoculum from the outer layer of papery onion skin to the papery layers below was evaluated. In all cases, counts were below the limit of detection for layers two and three; layer two was positive after enrichment for 2 of 12 samples immediately after inoculation and drying but not on stored onions (data not shown). Samples from layer three were never positive on enrichment; thus, only the outermost papery skin layer was analyzed in subsequent experiments. Three diluent volumes (3, 5, and 10 ml) were compared; no significant differences were observed for pathogen recovery. Thus, the lower volume (3 ml) was chosen for further studies to improve the limit of detection.

The survival of *Salmonella* and *E. coli* O157:H7 was evaluated after inoculation onto the outer surface of whole intact onions and was intended to mimic contamination that might occur on cured (dried) onions during distribution, retail display, and home storage. Counts on TSA (background mesophilic microbiota) for uninoculated controls ranged from undetectable (<1.5 log CFU per skin sample) to 3.2 log CFU per skin sample (each sample weighed approximately 1 g). For the uninoculated controls, no colonies were evident from the lowest dilution (one skin sample in 3 ml of peptone) plated onto any of the media containing R (detection limit: 1.5 log CFU per skin sample).

Salmonella cells in inocula prepared on agar plates are in a sessile form and can be significantly more desiccation tolerant than when the same strain is prepared in broth and in a planktonic state *(14, 24)*. The form of pathogens that might contribute to postharvest contamination of onion skin is unknown. The survival of *Salmonella* and *E. coli* O157:H7 that were cultured on TSAR or in TSBR were compared after inoculation onto the outer surface of whole intact onions. Significantly greater reductions were observed after 30 min of drying for *E. coli* O157:H7 and *Salmonella* cocktails that had been prepared in broth (2.8 and 2.6 log CFU per sample, respectively) compared with those collected from agar (0.6 and 0.9 log CFU per sample, respectively; Tables 1 and 2). At ambient condition, rates of decline were −2.4 and −0.39 log CFU per sample per day for *E. coli* O157:H7 prepared in broth or on agar, respectively (Table 3). Similar rates of decline were observed for *Salmonella* (−2.5 and −0.29 log CFU per sample per day for inoculum prepared in broth or on agar, respectively). More rapid declines were observed during storage at ambient conditions than at 4°C for those cultures prepared on agar but not in broth (Table 3). Onion skin inoculated with broth- or agar-prepared *E. coli* O157:H7 were negative by enrichment of some samples after 1 and 21 days of storage at 4°C, respectively (Table 1). Onion skins inoculated with broth-prepared *Salmonella* were negative by enrichment of some samples after 7 days of storage at 4°C; all samples inoculated with agar-prepared *Salmonella* were above the limit of detection by plating after 56 days of storage (Table 2). Populations of both pathogens at ambient conditions were below the limit of detection by plating for all but one sample by day 7 or 21 for broth- or agar-prepared inoculum.

Temperature recommendations for retail and consumer storage of bulb onions include cool (7 to 13°C) and dry, as

TABLE 1. *Survival of* E. coli *O157:H7 after inoculation onto the outer skin of whole yellow onions*[a]

Storage temp (°C)	Time postinoculation (h)	Storage time (day)	*E. coli* O157:H7 grown on plates			*E. coli* O157:H7 grown in broth		
			TSAR (log CFU/sample)[b]	SMACR (log CFU/sample)	Enrichment[c]	TSAR (log CFU/sample)	SMACR (log CFU/sample)	Enrichment
23	0		6.53 ± 0.10	6.30 ± 0.25	NA[d]	6.87 ± 0.32	6.55 ± 0.34	NA
		0	5.93 ± 0.29	5.41 ± 0.36	NA	4.11 ± 1.00	3.45 ± 0.76	NA
		1	4.77 ± 0.82	3.94 ± 0.86	NA	1.74 ± 1.04	1.76 ± 0.97	1/2
		7	2.96 ± 1.23	2.73 ± 0.80	0/1	0[e]	0	0/6
		14	0.78 ± 0.43	<0.4[f]	0/4	0.95	<0.4	0/5
		21	0.95	<0.4	0/5	0	0	0/6
		28	0	0	0/6	0	0	0/6
		42	0	0	0/6	ND[g]	ND	ND
		56	<0.4	<0.4	3/6	ND	ND	ND
4		1	4.97 ± 0.59	3.82 ± 0.94	NA	2.19 ± 0.97	0.87 ± 0.12	0/3
		7	4.42 ± 0.48	3.80 ± 0.90	NA	0.63 ± 0.21	<0.4	1/4
		14	3.71 ± 0.76	2.58 ± 0.67	NA	1.82 ± 0.49	0.48 ± 0	0/4
		21	3.88 ± 0.80	2.96 ± 0.27	0/1	0	0	0/6
		28	3.27 ± 0.59	1.48 ± 0.43	NA	0	0	0/6
		42	2.57 ± 1.21	1.32 ± 0.92	0/1	ND	ND	ND
		56	2.46 ± 1.83	1.92 ± 1.37	0/1	ND	ND	ND

[a] Onions dried for 0.5 h at ambient conditions and during storage at ambient conditions or 4°C ($n = 6$).
[b] Values are means ± standard deviations. A sample is a circle (3.3 cm in diameter) of onion skin cut from the outer layer.
[c] Number of samples positive for *E. coli* O157:H7 as detected by enrichment/total number of samples analyzed. Samples were only analyzed when no colonies were detected on the agar plates.
[d] NA, not applicable.
[e] No colonies, detected by plating; no samples positive on enrichment.
[f] Samples below limit of detection (0.4 log CFU per sample).
[g] ND, not done.

well as room temperature *(20)*. *E. coli* O157:H7 and *Salmonella* collected from agar declined by 0.39 and 0.29 log CFU per sample per day, respectively, at ambient laboratory temperature (23 ± 0.3°C); if reduction kinetics are linear, a 5-log reduction would be predicted to occur within 2 and 3 weeks, respectively (Table 3). The rates of reduction were 0.08 and 0.06 log CFU per sample per day at 4°C; so, assuming linearity, a 5-log reduction would be predicted in 9 and 12 weeks of refrigerated storage for *E. coli* O157:H7 and *Salmonella,* respectively. The shelf life of bulb onions is 24 to 36 weeks under optimal postcuring storage (0°C) and 65 to 70% RH *(23)*. Survival of *E. coli* O157:H7 and *Salmonella* was not determined at 0°C.

Growth of *E. coli* O157:H7 and *Salmonella* on freshly diced and commercial fresh-cut yellow onions. Fresh-cut onions obtained from retail markets are likely to have been prepared one or more days before purchase. Survival of *E. coli* O157:H7 and *Salmonella* was compared on laboratory-prepared freshly diced onions and two different brands (denoted brand A and B) of commercial fresh-cut onions during storage in closed containers at ambient conditions, because a single brand was not available at retail for both replicates of the experiment. Few (one or two) or no colonies were observed on TSAR, SMACR, and BSAR at the lowest dilution plated for all uninoculated diced onion controls (<1.3 log CFU/g). None of the occasional colonies that were observed displayed typical characteristics of the inoculated bacteria on the agar containing R. The levels of *Salmonella* in homogenized chopped onions were compared with 0.1% peptone controls. No decline of *Salmonella* was observed in the homogenized onions or peptone control held at ambient conditions for 1 h (data not shown).

The levels of native microbiota in uninoculated freshly diced controls (plated onto TSA) were 2.3 log CFU/g initially and increased to 8.1 log CFU/g at 36 h of ambient storage. In the commercially prepared onions, the initial levels of native microbiota were 3.0 and 5.4 log CFU/g for brand A and brand B, respectively, on TSA. After 36 h, levels increased to 6.9 and 8.2 log CFU/g for brand A and brand B, respectively.

No significant growth was observed for either pathogen for the freshly diced samples over the first 8 h of ambient storage (Fig. 1). Populations of both pathogens increased from 3 to over 6 log CFU/g within 24 h (Fig. 1) in the freshly diced onion; growth rates of 0.28 and 0.31 log CFU/g/h were determined for *E. coli* O157:H7 and *Salmonella,* respectively. Very different results were observed for the two brands of commercially prepared onions. For brand A, growth of 1.7 and 2.4 log CFU/g was observed for *E. coli* O157:H7 and *Salmonella,* respectively, after 14 h, whereas little to no growth was observed in brand B over the same time. Total increases of 2.7 and 2.4 log CFU/g were observed for *E. coli* O157:H7 and *Salmonella,* respectively, over 36 h in brand A (Fig. 1); populations declined or did not change in brand B. The inconsistency in results with commercially prepared product may have been due to the age and physical condition

TABLE 2. *Survival of* Salmonella *after inoculation onto the outer skin of whole yellow onions*[a]

Storage temp (°C)	Time postinoculation (h)	Storage time (day)	*Salmonella* grown on plates: TSAR (log CFU/sample)[b]	*Salmonella* grown on plates: BSAR (log CFU/sample)	*Salmonella* grown on plates: Sample size (*n*)	*Salmonella* grown on plates: Enrichment[c]	*Salmonella* grown in broth: TSAR (log CFU/sample)	*Salmonella* grown in broth: BSAR (log CFU/sample)	*Salmonella* grown in broth: Sample size (*n*)	*Salmonella* grown in broth: Enrichment
23	0		6.86 ± 0.11	6.82 ± 0.13	9	NA[d]	7.03 ± 0.12	6.96 ± 0.08	9	NA
		0	5.94 ± 0.59	5.71 ± 0.66		NA	4.41 ± 0.71	3.89 ± 0.87		NA
		1	4.19 ± 0.71	3.80 ±0.85		NA	1.64 ± 0.75	1.47 ± 0.53		NA
		7	2.61 ± 1.22	2.49 ± 0.95		0/1	0.26 ± 0.45	<0.4[e]		0/8
		14	1.38 ± 0.70	1.02 ± 0.39		0/4	<0.4	<0.4		1/9
		21	1.08	<0.4		0/8	0[f]	0		0/9
		28	<0.4	<0.4		1/9	0	0		0/9
		42	<0.4	<0.4		2/9	0	0	3	0/3
		56	<0.4	<0.4	7	0/7	0	0	3	0/3
4		1	5.35 ± 0.30	4.93 ± 0.62	6	NA	1.89 ± 0.64	1.43 ± 0.61	6	NA
		7	4.85 ± 0.51	4.51 ± 0.70		NA	1.67 ± 1.26	1.29 ± 1.15		2/4
		14	4.45 ± 0.50	3.85 ± 0.80		NA	1.53 ± 0.14	0.93 ± 0.64		0/4
		21	4.13 ± 0.31	3.34 ± 0.58		NA	1.34 ± 0.12	0.98 ± 0.28		0/4
		28	3.85 ± 0.54	3.25 ± 0.87		NA	1.38 ± 0.60	0.90 ± 0.60		0/4
		42	2.92 ± 1.03	2.36 ± 1.31		NA	ND[g]	ND		ND
		56	2.50 ± 1.22	2.36 ± 1.24		NA	ND	ND		ND

[a] Onions dried for 0.5 h at ambient conditions and during storage at ambient conditions or 4°C.
[b] Values are means ± standard deviations. A sample is a circle (3.3 cm in diameter) of onion skin cut from the outer layer.
[c] Number of samples positive for *Salmonella* as detected by enrichment/total number of samples analyzed. Samples were only analyzed when no colonies were detected on the agar plates.
[d] NA, not applicable.
[e] Samples below limit of detection (0.4 log CFU per sample).
[f] No colonies, detected by plating; no samples positive on enrichment.
[g] ND, not done.

TABLE 3. *Growth or decline rates and lag times for* E. coli *O157:H7 and* Salmonella *inoculated onto skins of whole onions and freshly diced onions and stored at either ambient conditions or 4°C*

Onion sample	Culture preparation	Organism	Temp	Inoculation method[a]	Rate of change[b]	Rate unit	Lag time (h)	R^2
Skin	Plate	*E. coli* O157:H7	Ambient	Direct spot (Table 1)	−0.39	log CFU/sample/day	None[c]	0.84
			4°C	Direct spot (Table 1)	−0.079	log CFU/sample/day	None	0.56
		Salmonella	Ambient	Direct spot (Table 2)	−0.29	log CFU/sample/day	None	0.69
			4°C	Direct spot (Table 2)	−0.056	log CFU/sample/day	None	0.69
	Broth	*E. coli* O157:H7	Ambient	Direct spot (Table 1)	−2.4	log CFU/sample/day	None	0.58
			4°C	Direct spot (Table 1)	−1.9	log CFU/sample/day	None	0.63
		Salmonella	Ambient	Direct spot (Table 2)	−2.5	log CFU/sample/day	None	0.71
			4°C	Direct spot (Table 2)	−2.4	log CFU/sample/day	None	0.76
Diced	Broth	*E. coli* O157:H7	Ambient	Direct (Fig. 1)[d]	0.28	log CFU/g/h	8.7	0.95
			Ambient	Direct (Fig. 3)	0.22	log CFU/g/h	6.1	0.98
			Ambient	Direct combined (Figs. 1 and 3)	0.25	log CFU/g/h	7.5	0.95
			Ambient	Indirect (Fig. 3)	0.19	log CFU/g/h	None	0.57
		Salmonella	Ambient	Direct (Fig. 1)	0.31	log CFU/g/h	8.8	0.92
			Ambient	Direct (Fig. 3)	0.27	log CFU/g/h	7.2	0.96
			Ambient	Direct combined (Figs. 1 and 3)	0.29	log CFU/g/h	8	0.94
			Ambient	Indirect (Fig. 3)	0.19	log CFU/g/h	None	0.79

[a] Freshly diced onions were inoculated with prepared inoculum (direct method), and then after 12 h of ambient storage, some of the initially inoculated onions were mixed 1:9 (wt/wt) with refrigerated uninoculated freshly diced onion (indirect method).

[b] Individual data points from TSAR were analyzed.

[c] None, no lag time identified.

[d] Figure mentioned shows the bacterial growth curves associated with the specific trial used to determine rate of change.

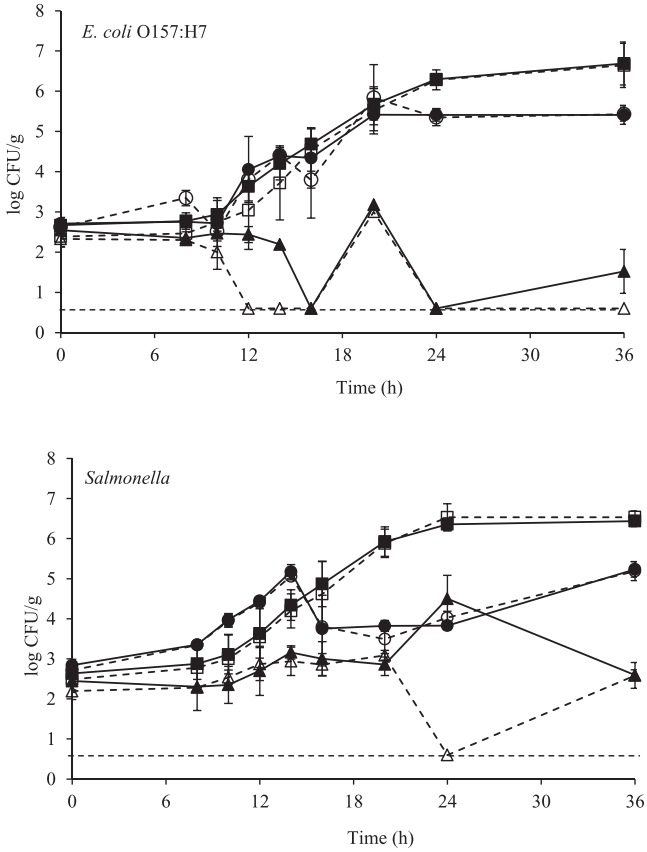

FIGURE 1. *Growth of* E. coli *O157:H7 and* Salmonella *cocktails on freshly diced yellow onions (squares,* n = *6) and two brands of commercial fresh-cut diced yellow onions (brand A: circles,* n = *3; brand B: triangles,* n = *3) stored in closed containers held at ambient conditions; samples plated on TSAR (solid lines and closed symbols) and either BSAR or SMACR (dashed lines and open symbols). Dashed line indicates limit of detection (TSAR, 0.6 log CFU/g).*

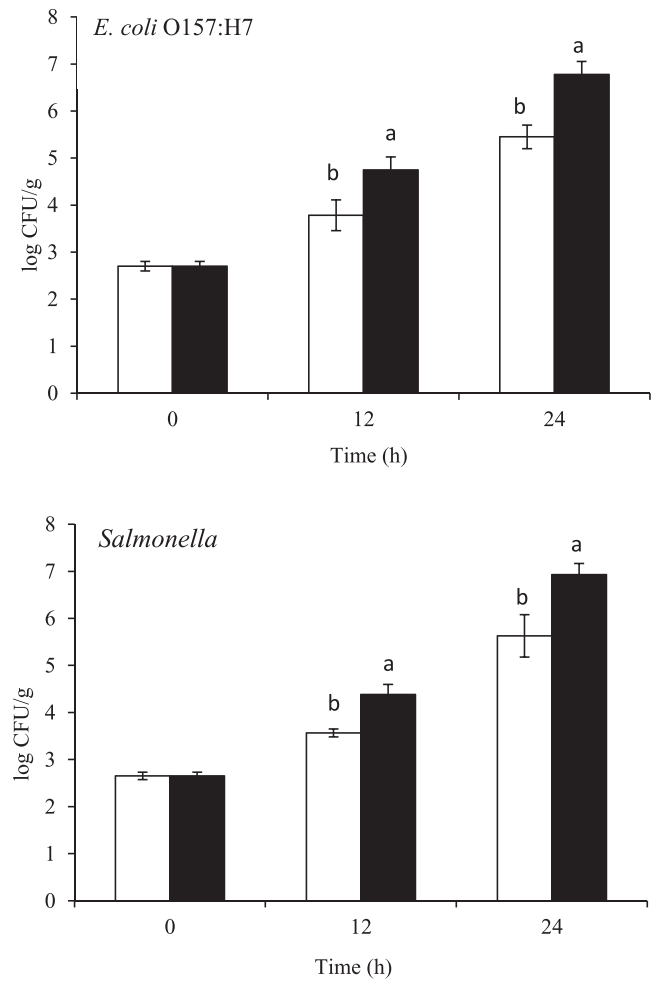

FIGURE 2. *Growth of* E. coli *O157:H7 and* Salmonella *cocktails on freshly diced yellow onions stored in open or closed containers (open and solid bars, respectively) held at ambient conditions (TSAR,* n = *6). Comparisons of the mean log CFU per gram values were made between open and closed cups for both bacteria types. Within storage time, means with different letters are significantly different (*P < *0.05).*

of the product and the type and levels of background microbiota. Laboratory-prepared diced onions were used for subsequent experiments due to this variation.

Growth of *E. coli* O157:H7 and *Salmonella* on freshly diced onions kept in open or closed containers at ambient conditions. The growth of *E. coli* O157:H7 and *Salmonella* was compared in freshly diced onion (initial pH 5.6) stored under ambient conditions for up to 24 h in open or closed containers. The measured volume of the container was 220 $\pm$ 3 cm^2 with 28 $\pm$ 3 cm^2 headspace after the addition of onions. The initial levels of native microbiota were 0.65 log CFU/g on TSA, increasing to 6.0 and 6.8 log CFU/g for the open and closed containers, respectively. Significantly greater increases in populations of *E. coli* O157:H7 and *Salmonella* were observed in onions that were stored in closed containers (Fig. 2). *E. coli* O157:H7 populations increased on freshly diced onions by 1.1 and 2.8 log CFU/g in open containers and by 2.0 and 4.1 log CFU/g in closed containers, after storage for 12 and 24 h, respectively. *Salmonella* populations increased by 0.92 and 3.0 log CFU/g in open containers and by 1.7 and 4.2 log CFU/g in closed containers, after storage for 12 and 24 h, respectively. Onions stored in open containers were visibly drier than those stored in closed containers, and the reduced moisture may have impacted growth. Inoculated diced onions were stored in closed containers for all further experiments.

Survival of *E. coli* O157:H7 and *Salmonella* on freshly diced onions stored in closed containers at 4°C. Diced onions are typically stored under refrigerated conditions at retail and in the home. If commercially prepared diced onions are stored optimally (4°C and controlled atmosphere of 2% O_2 and 10% CO_2), the shelf life is approximately 14 days *(2)*. Populations of both pathogens in freshly diced onions decreased by less than 0.5 log CFU/g over 6 days of storage at 4°C. Levels of native microbiota determined on TSA that were incubated at 37°C increased from 4.3 to 5.0 log CFU/g after 6 days at 4°C in the uninoculated controls. We did not determine levels of native psychrotrophic populations.

Growth of *E. coli* O157:H7 and *Salmonella* on freshly diced onions mixed with ambient-stored diced onions inoculated 12 h previously. Onions are peeled, and

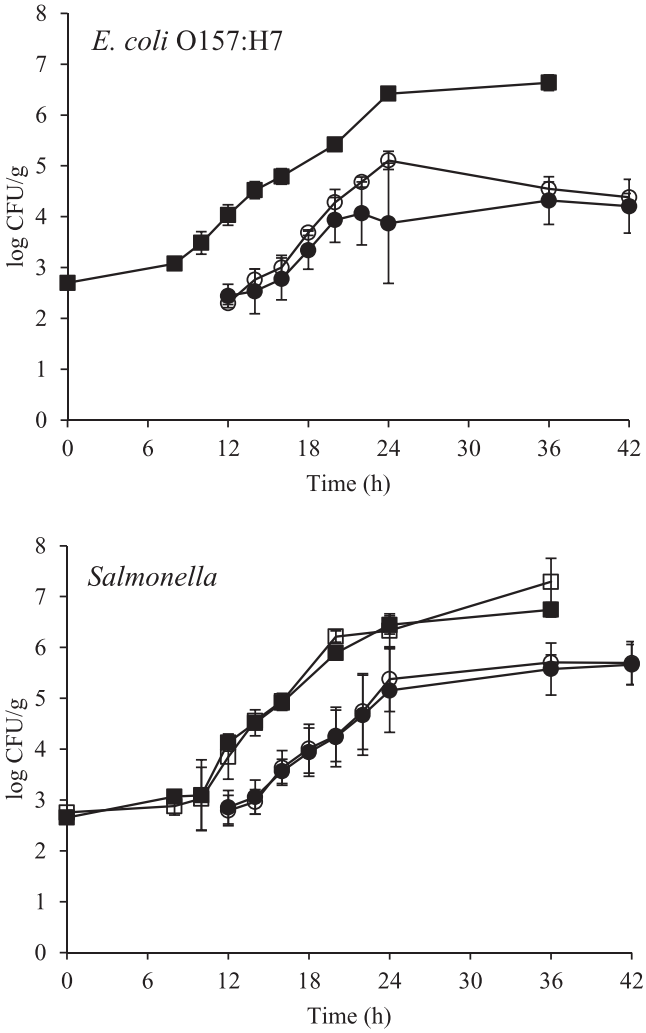

FIGURE 3. *Growth of* E. coli *O157:H7 and* Salmonella *on freshly diced yellow onions stored in closed containers held at ambient conditions; samples plated on TSAR (closed squares) and BSAR (open squares) (*n = 6*). After 12 h of storage, some of the initially inoculated onions were mixed with cold uninoculated freshly diced yellow onions at 1:9 (wt/wt); samples plated on TSAR (closed circles,* n = 6*), SMACR (*E. coli*, open circles,* n = 3*), or BSAR (*Salmonella*, open circles,* n = 6*).*

the skin is discarded during preparation. Although this practice might serve to remove or reduce contaminants, if present on the skin, it does not preclude transfer of organisms to the edible flesh during preparation *(17, 21, 25)*. Foodborne pathogens can adhere to surfaces used in typical food preparation and may contribute to cross-contamination of ready-to-eat products prepared in the same area *(6, 13, 21)*. The transfer of *L. monocytogenes* from a single inoculated onion was sufficient to contaminate the next 20 uninoculated onions that were sliced using the same commercial slicer *(22)*. Surfaces containing food residue may allow for enhanced survival of contaminants *(16)*.

An investigation into a 2008 outbreak of *E. coli* O157:H7 gastroenteritis revealed that onions were stored at room temperature and were topped up as needed from partial bowls or a central container that was stored in the cooler *(21)*. The stated practice in the outbreak-associated restaurant was to discard any onions that had been held at room temperature at the end of each day. Records indicated that staff reported to work as early as 7:00 a.m. and left as late at 8:30 or 9:00 p.m., but it was unclear whether diced onions would have been held at ambient temperature over this entire 13- to 14-h period. In the current study, the levels of *E. coli* O157:H7 and *Salmonella* increased from 2.7 to 4 log CFU/g (Fig. 3) after 12 h of storage at room temperature. At this point the inoculated onions were combined 1:9 (wt/wt) with refrigerated uninoculated freshly diced onions; the levels of *E. coli* O157:H7 and *Salmonella* in the mixed samples were 2.4 and 2.9 log CFU/g, respectively. After a further 12 h of ambient storage, *E. coli* O157:H7 and *Salmonella* populations increased by 1.5 and 2.3 log CFU/g, respectively, to levels of 3.9 and 5.2 log CFU/g, respectively. The populations increased by a further 0.3 to 0.5 log CFU/g after 24 to 30 h of storage. In both cases, the maximum populations were lower than maximum populations achieved in the initially inoculated onions. Initial levels of native microbiota plated on TSA were 4.2 log CFU/g and increased to 9.1 log CFU/g over 36 h.

Growth rates of *E. coli* O157:H7 and *Salmonella* on directly and indirectly inoculated freshly diced onions. Growth rates determined for *E. coli* O157:H7 and *Salmonella* in freshly diced onions stored at ambient conditions were 0.22 and 0.27 log CFU/g/h, respectively (direct inoculation, Table 3). When mixed with uninoculated chopped onions (i.e., indirect inoculation), the growth rates were 0.19 CFU/g/h. Predicted lag times were 6 and 7 h for *E. coli* O157:H7 and *Salmonella,* respectively, in unmixed samples; no lag was observed for either pathogen in the mixed (topped-off) samples. The difference in lag time is relevant to food service scenarios in which topping off may be a standard practice. The maximum holding time for food for sale or service above 5°C is 4 h in accordance with U.S Food Code Section 3-501.19(B) *(27)*. The 4-h holding time should be measured from the time the chopped onions were initially exposed to ambient conditions.

Onion skins might be contaminated before harvest from irrigation water or soil, during or after harvest from contaminated equipment, or by human handling or during final preparation. The physiological state of pathogens under these various contamination scenarios is not well understood. Survival of both pathogens was enhanced on onion skin when the inoculum was grown on solid medium than when cultured in broth. Culture preparation is one of the factors to consider when inoculating food products *(19)*, and the data presented here and elsewhere *(14, 24)* suggest that for circumstances in which desiccation may occur, collection of inoculum from agar represents a worst-case or conservative experimental approach. *E. coli* O157:H7 and *Salmonella* populations declined on the outer skin of whole intact onions at both ambient conditions and 4°C.

Populations of *E. coli* O157:H7 and *Salmonella* did not decline at 4°C and increased by 0.2 to 0.3 log CFU/g/h at ambient conditions in diced onions. Lag times of 6 to 9 h were observed with freshly inoculated onion; no lag was

observed when previously inoculated and uninoculated onions were mixed. A difference in growth of both pathogens was noted between freshly diced onion and two commercial brands of fresh-cut onion. Determining the reasons for these differences was beyond the scope of this study but may be worthy of further investigation. Diced onion is appropriately considered a perishable product and should be kept refrigerated during distribution and in the home. Food service establishments should evaluate the practice of topping off condiments, such as diced onion, that are held at ambient conditions.

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