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Fate of inoculated *Listeria monocytogenes* on yellow onions (*Allium cepa*) under conditions simulating food service and consumer handling and storage

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ABSTRACT

Recalls and cases of listeriosis have been associated with the presence of *Listeria monocytogenes* in fresh-cut refrigerated or frozen onions or associated processing environments. The survival or growth of *L. monocytogenes* on the outer surface of whole onions and in diced onion was evaluated during simulated retail or consumer storage. Whole and diced yellow onions (*Allium cepa*) were inoculated with a 6-strain cocktail of *L. monocytogenes* collected either from agar plates or from broth culture. Marked circles (3.3 cm in diameter) on the outer papery skin of whole onions were spot inoculated (10 µl) at 7 log CFU per circle, dried for 30 min and then stored at 4 or 23 °C for up to 8 weeks. The marked circles or "disks" of the outermost skin layer were excised for sampling. Diced onions were inoculated at 3 log CFU/g and then stored in closed containers at 4 or 10 °C for 28 or 21 days, respectively, or at 23 °C for 38 h. Populations of *L. monocytogenes* were determined by plating each sample onto both tryptic soy agar and modified Oxford or CHROMagar Listeria agars. At 4 °C, populations of *L. monocytogenes* on whole onion declined from initial pre-drying levels of 6–7 log CFU/disk to mean levels of 2.39 ± 1.14 log CFU/disk at week 8; at 23 °C, populations declined to below the limit of detection by plating (< 0.40 log CFU/disk) and by enrichment in 3, 9, and 12 of 12 samples at weeks 2, 3, and 4, respectively. No significant change in *L. monocytogenes* populations was observed in diced onion during 28 days of storage at 4 °C. A maximum rate of change of 0.0081 log CFU/g/day; a mean *L. monocytogenes* population of 6.86 ± 0.44 log CFU/g was observed at 17 days of storage at 10 °C. At 23 °C calculated lag times of 4.7 and 8.6 h, maximum rates of change of 0.15 and 0.16 log CFU/g/h, and maximum *L. monocytogenes* populations of 6.24 ± 0.07 and 6.10 ± 0.03 log CFU/g after 38 h of storage were observed in diced onion inoculated with agar and broth cultures, respectively. Diced onions support the growth of *L. monocytogenes* at 10 and 23 °C but not at 4 °C.

1. Introduction

The bulb onion (*Allium cepa*) is commercially available in a wide range of forms, including whole, fresh cut (e.g., diced, rings, sliced, slivered, and pureed), and frozen (e.g., diced, rings [plain or battered], and whole pearl). A shelf life of up to 9 months can be expected for sufficiently dried, whole onions stored near 0 °C and below 65–70% relative humidity (RH) (Suslow, 1996). Fresh-cut onions can have a shelf life of ~14 days when packaged under appropriate modified atmosphere (at least 2% O_2 and 10% CO_2) and stored at 4 °C (Blanchard, Castaigne, Willemot, & Makhlouf, 1996).

Onions are most often consumed as ingredients in either cooked or uncooked savory dishes. An outbreak of *Escherichia coli* O157:H7 gastroenteritis was linked to consumption of uncooked diced onions used as a garnish for hamburgers at a fast food chain restaurant (NBPSDHU, 2009). While outbreaks of listeriosis have not been associated with fresh onion consumption, isolation of *Listeria monocytogenes* from product and environmental samples has led to recalls of fresh-cut onions alone or in vegetable mixtures (CDPH, 2012; FDA, 2012, 2016a). Frozen vegetables, including frozen chopped onions, were implicated in a multistate outbreak of listeriosis (CDC, 2016; FDA, 2016b).

On the skins of whole unpeeled onions, *E. coli* O157:H7 and *Salmonella* populations declined by 0.4 and 0.3 log CFU per day, respectively, at ambient conditions, and by 0.08 and 0.06 log CFU per day, respectively, at 4 °C (Lieberman et al., 2015). The survival of *L. monocytogenes* on the skins of whole unpeeled onions has not been evaluated. *L. monocytogenes* survived but did not grow on whole peeled onions held at 4 °C for up to 7 days (Scollon et al., 2016), on sliced

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onions held in modified-atmosphere packages (MAP) at 4 °C for up to 9 days (Farber, Wang, Cai, & Zhang, 1998), and in chopped onions held at 5 °C for 14 days (Salazar et al., 2017). Growth of cocktails or individual strains of *L. monocytogenes* was observed in fresh-cut onions at 10 °C for 9 or 14 days (Farber et al., 1998 or Salazar et al., 2017, respectively) and at 25 °C for 7 days (Salazar et al., 2017). In the current study, the fate of a 6-strain cocktail of *L. monocytogenes* was evaluated on the skin of whole unpeeled onions stored at 4 and 23 °C for up to 2 months, and on diced onions stored at 4 and 10 °C for 28 and 21 days, respectively, or at 23 °C for 38 h, to simulate a range of retail or food service and consumer handling conditions.

2. Materials and methods

2.1. Onion samples

Whole yellow onions (*Allium cepa*) with smooth outer skins were purchased from local retail stores (Davis, CA) and stored for up to 1 day at ambient conditions (23.4 ± 0.4 °C, 45.4% ± 11.1% relative humidity [RH]) before use. While the stock keeping unit was the same for each purchase, the specific cultivar and age of the onions was not known.

2.2. Bacterial cultures

Six strains of *L. monocytogenes* were received from the U.S. Food and Drug Administration (College Park, MD): LIS0234 and LIS0235, isolated from raw diced yellow onions associated with a recall of pre-packaged diced yellow onions (FDA, 2012); LIS0133, an environmental isolate from a fresh-cut celery processing facility associated with an outbreak of listeriosis (Gaul et al., 2013); and isolates associated with a multi-state cantaloupe-associated outbreak (CDC, 2012), including LIS0110, isolated from whole cantaloupes, and LIS0087 and LIS0077 isolated from within the associated cantaloupe packing facility. A cocktail of these six parent strains was used in experiments with whole onions.

In preliminary experiments, the background microbiota in the diced onions interfered with enumeration of the *L. monocytogenes* inoculum on both tryptic soy agar and CHROMagar Listeria. A stepwise procedure (Parnell et al., 2005) was used to isolate rifampin-resistant mutants of each parental strain listed above, and a separate cocktail of these six rifampin-resistant strains was used in the experiments with diced onions. Growth of the parent and mutant strains was not significantly different when compared in tryptic soy broth (TSB) at 37 °C (data not shown). All cultures were stored at −80 °C in TSB supplemented with 15% glycerol. Unless otherwise specified, culture media were Difco brand (BD, Franklin Lakes, NJ).

2.3. Preparation of inocula

Individual frozen stock cultures were streaked onto tryptic soy agar (TSA) or TSA with 50 μg/ml of rifampin (TSAR) and incubated overnight at 37 °C. Isolated colonies were transferred into 10 ml of TSB or TSB with 50 μg/ml of rifampin (TSBR) and incubated overnight at 37 °C. For the inoculum collected from agar plates, 1 ml from the second overnight culture was plated onto 150 × 15 mm TSA or TSAR plates and incubated at 37 °C for 24 h. Cell lawns were collected from plates by adding 9 ml of sterile ultra-pure water (Milli-Q Advantage A10, MilliporeSigma, Burlington, MA) to each plate and scraping with an L-shaped spreader. A 6-strain (agar-prepared) *Listeria* cocktail was prepared by combining equal amounts (5 ml) of each strain. For the broth-prepared *Listeria* inoculum, 1 ml of each of the second overnight TSBR cultures was centrifuged at 16,000 × *g* for 2 min. The supernatant was discarded and cells were washed twice with 1 ml of 0.1% peptone and then suspended in 1 ml of sterile ultra-pure water. A 6-strain (broth-prepared) *Listeria* cocktail was made by combining equal amounts (200 μl) of each strain. Dilutions were made in sterile ultra-pure water to achieve initial target populations.

2.4. Onion preparation and inoculation

Whole onions with intact outer papery skins were prepared and inoculated as described in Lieberman et al. (2015). Briefly, four to five circles or "disks" (3.3-cm diameter, 9-cm^2 area) were drawn on the outermost papery surface of whole yellow onions to define the areas to be inoculated. The agar-prepared *L. monocytogenes* inoculum (10 1-μl drops) was deposited inside the circle to give an initial target level of ~7 log CFU per disk. Whole yellow onions were held in a biosafety cabinet for 30 min to allow the inoculum to dry and were then transferred to the appropriate storage temperature.

Diced onions were prepared by peeling whole onions and cutting them into quarters with a knife. Each quartered section was then placed in a manual vegetable chopper (Vidalia Chop Wizard, National Express Online, Norwalk, CT), and diced into pieces approximately 1 × 1 cm. Diced onions (20 g) were placed into 240-ml specimen storage containers (Thermo Fisher Scientific, Waltham, MA) and 20 μl of the prepared *L. monocytogenes* inoculum (either agar- or broth-prepared) was added to target an initial level of 3 log CFU/g. To distribute the inoculum, the closed storage containers with inoculated onions were shaken in a 30° arc for 25 s. Agar and broth-prepared inocula were compared on diced onions stored at ambient conditions (23 °C). For all other experiments, agar-prepared inoculum was used.

2.5. Storage conditions

After inoculation, whole yellow onions were stored at either refrigeration (target 4 °C) for 8 weeks or ambient temperature (target 23 °C) for 4 weeks. Microbial populations were determined immediately after inoculation, after holding for 30 min in a biosafety cabinet to dry the inoculum (time zero of storage), after 24 h, and every 1–2 weeks.

After inoculation, diced onions were stored in closed specimen containers at refrigeration (target 4 °C), cool (target 10 °C), or ambient temperature (target 23 °C) for 28 days, 21 days, or 38 h, respectively. Microbial populations were determined as follows: immediately following inoculation and every 3–4 days up to day 28 or day 21 for samples stored at 4 or 10 °C, respectively, and at 7, 14, 16, 18, 20, 22, 24, 30, and 38 h after storage for samples stored at 23 °C.

Water activity and pH of uninoculated control samples were measured at every time point when samples were stored at 4 and 10 °C, and at 0, 14, 24, and 38 h for samples stored at 23 °C. The water activity was determined with an Aqua Lab 4 TE Duo (Decagon Devices, Pullman, WA). For pH determination, diced onion (20 g) was mixed with 4 ml of ultra-pure water in a 200-ml Whirl-Pak filter bag (Nasco, Modesto, CA) and then homogenized by pushing a rolling pin back and forth over the bag; the pH of the homogenate was determined using an Orion PerpHec T meter (Thermo Fisher Scientific, Waltham, MA).

Samples stored at 4 and 10 °C were held in refrigerated incubators (Revco, Thermo Fisher Scientific). Samples stored at ambient condition were held in large plastic bins on a laboratory bench. Temperature and RH of each storage area were monitored with data loggers (TempTale 4, Sensitech Inc., Beverly, MA). Temperature and RH inside the specimen containers containing diced onions were monitored with iButton data loggers (Maxim Integrated, San Jose, CA).

2.6. Bacterial recovery and enumeration

Onion samples were processed as previously described by Lieberman et al. (2015). At each sampling point, the inoculated marked circles on whole onions were aseptically excised by cutting and removing the outermost papery skin layer with a sterile scalpel. Each excised circle or "disk" sample, which equated to approximately 1 g of onion skin, was placed into a separate 120-ml Whirl-Pak bag and combined with 3 ml of 0.1% peptone. Sample bags were shaken by