Listeria monocytogenes Transfer during Mechanical Dicing of Celery and Growth during Subsequent Storage

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ABSTRACT

The transfer of Listeria monocytogenes to previously uncontaminated product during mechanical dicing of celery and its growth during storage at various temperatures were evaluated. In each of three trials, 275 g of retail celery stalks was immersed in an aqueous five-strain L. monocytogenes cocktail to obtain an average of 5.6 log CFU/g and then was diced using a hand-operated dicer, followed by sequential dicing of 15 identical 250-g batches of uninoculated celery using the same dicer. Each batch of diced celery was examined for numbers of Listeria initially and after 3 and 7 days of storage at 4, 7, and 10°C. Additionally, the percentage by weight of inoculated product transferred to each of 15 batches of uninoculated celery was determined using inoculated red stems of Swiss chard as a surrogate. Listeria transfer to diced celery was also assessed after removing the Swiss chard. L. monocytogenes transferred from the initial batch of inoculated celery to all 15 batches of uninoculated celery during dicing, with populations decreasing from 4.7 to 1.7 log CFU/g. Storing the diced celery at 10°C yielded a L. monocytogenes generation time of 0.87 days, with no significant growth observed during storage at 4 or 7°C. Consequently, mitigation strategies during dicing and proper refrigeration are essential to minimizing potential health risks associated with diced celery.

As health awareness in the United States grows, consumers are becoming increasingly interested in maintaining healthy diets supported by fresh fruits and vegetables. However, due to contamination in the field, cross-contamination during harvesting and processing, and ineffective microbial reduction strategies, fresh produce can serve as vectors for pathogens. During 2009 and 2010, 27 outbreaks, including 1,259 illnesses, resulted from ingestion of vegetables contaminated with microbial pathogens (9). Among the bacterial foodborne pathogens responsible for these outbreaks, Listeria monocytogenes causes the third highest number of deaths, estimated at 255 each year (8).

Various surveys of retail establishments (12, 20, 35) and processing facilities (1, 21) have shown L. monocytogenes to be an uncommon contaminant of fresh vegetables, indicating that fresh produce presents a low risk for transmission of listeriosis to consumers (1). However, L. monocytogenes has occasionally been identified in ready-to-eat salads containing raw celery prepared at supermarkets (10), as well as in other varieties of ready-to-eat salads (20, 23, 32), consequently posing a risk for consumers.

In 1979, an outbreak of systemic listeriosis involving 23 people was epidemiologically linked to consumption of raw celery, tomatoes, and lettuce (19). Nine years later, an outbreak of gastroenteritis at the U.S. Air Force Academy originally attributed to Salmonella was later traced to diced celery from chicken salad that had been washed and stored in nonpotable water contaminated with norovirus (41). More recently, in 2010, 10 listeriosis cases, including five deaths, were traced to contaminated diced celery from a produce processing facility in San Antonio, TX (38), which had been used as an ingredient in chicken salad that was served to hospital patients (17).

Outbreaks such as these can result from microbial contamination of fresh produce at any point from farm to fork. During harvesting, Listeria may contaminate produce via decaying vegetation, feces, soil, sewage effluents (2), or through dissemination by animals, such as wild birds (13). Worker handling during harvest and postharvest processing may also lead to contamination if inadequate hygienic practices are followed (4). During postharvest processing, produce is prone to contamination from other products, wash water, or processing equipment, allowing the spread of microbial pathogens (33). Postprocessing storage at
improper temperatures may then permit growth of microbial pathogens to levels potentially hazardous to consumers (42).

Past studies have quantified bacterial transfer during the processing of leafy greens (5, 6), slicing of delicatessen meats (28, 40), and production of ground beef (15, 16). Additionally, the impact of storage time and temperature on produce contaminated with *Listeria* spp. has been studied in frozen vegetables (27), shredded cabbage (26), bagged leafy greens (42), cooked sweet corn and bean sprouts (34), as well as in refrigerated diced (29) and fresh-cut celery sticks (39). Whereas processing may allow for the transfer of low numbers of pathogens to previously uncontaminated product, these same pathogens may reach potentially hazardous levels during subsequent storage, particularly under abusive conditions. Consequently, the objective of this study was to quantify the transfer of *L. monocytogenes* from inoculated celery to uncontaminated celery during mechanical dicing and to assess growth of the pathogen in celery at commonly encountered home storage temperatures.

**MATERIALS AND METHODS**

**Experimental design.** For this study, retail celery stalks inoculated with a five-strain cocktail of *L. monocytogenes* were diced, followed by dicing of uninoculated celery, to quantify *L. monocytogenes* transfer during mechanical dicing. The spread of contamination during dicing was also assessed using stalks of Swiss chard, a colored surrogate for inoculated celery. Thereafter, populations of *L. monocytogenes* were assessed in diced celery during storage at 4, 7, and 10°C.

**Produce.** Individually bagged heads of celery (*Apium graveolens*) were purchased from a local retailer, and locally grown Swiss chard (*Beta vulgaris var. cicla*) was obtained from an area farmers’ market. Upon arrival, all celery and Swiss chard was stored in a 4°C walk-in cooler and was used within 3 days of purchase. Stalks with intact surfaces free of damage were cut into pieces 5 cm in length immediately before inoculation and dicing.

**Bacterial strains.** Stock cultures of five outbreak-related strains of *L. monocytogenes* (J1-177, serotype 1/2b [human sporadic case, 1997]; J1-110, serotype 4b [Mexican style soft cheese, Los Angeles, CA, 1985]; N3-008, serotype 4b [coleslaw, Halifax, Nova Scotia, Canada, 1981]; N3-031, serotype 1/2a [hot dog, United States]; R2-499, serotype 1/2a [sliced turkey, 2000]) were obtained from Dr. Martin Wiedmann (Cornell University, Ithaca, NY). All strains were prepared in Trypticase soy broth (Difco, BD, Sparks, MD) containing 0.6% (wt/vol) yeast extract (TSBYE; Difco, BD) to which 10% (vol/vol) glycerol (Sigma Chemical Co., St. Louis, MO) was added for storage at −80°C.

**Inoculation.** Each strain was streaked from the frozen stock culture onto Trypticase soy agar plates (Difco, BD) containing 0.6% (wt/vol) yeast extract and was incubated at 37°C for 24 h. An isolated colony of each strain was subcultured twice in 9 ml of TSBYE at 37°C for 24 h before use. The five cultures were combined in equal volumes to obtain a 45-mL cocktail, 15 ml of which was diluted 1:100 in 1,500 ml of distilled water (−15°C) to contain 7 log CFU/ml. Two batches of celery stalks for the *Listeria* transfer (275 ± 0.5 g) and growth study (600 ± 0.5 g), and one batch of red Swiss chard stems for the *Listeria* transfer study (275 ± 0.5 g), were immersed in the *L. monocytogenes* suspension for 1 h, removed using sterilized tongs, and air dried for 30 min at 22°C in a biosafety cabinet to give populations of 5 and 6 log CFU/g for celery and Swiss chard, respectively; these populations were confirmed by direct plating as detailed below.

**Celery dicing and storage.** One 250-g batch of un inoculated celery stalks 5 cm in length was diced using a hand-operated Easy Dicer (model N55100E, Nemco Inc., Hicksville, OH), followed by dicing of 250 g of previously inoculated celery and 15 consecutive 250-g batches of un inoculated celery. After exiting the dicer, each batch of celery was collected in a sterile Whirl-Pak bag (Nasco, Fort Atkinson, WI) for enumeration of *L. monocytogenes* within 2 h. Following removal of samples for initial *L. monocytogenes* enumeration, each batch of diced celery was evenly divided into three sterile Whirl-Pak bags, which were subsequently closed and stored for 7 days at 4, 7, or 10°C. Samples held at each of the three temperatures were quantitatively examined for *L. monocytogenes* after 3 and 7 days of storage.

**Dicing of Swiss chard followed by celery.** Dicing operations were performed as previously described, beginning with 250 g of uninoculated celery stalks. Thereafter, 250 g of inoculated red Swiss chard stalks, which served as a colored surrogate for celery, was diced, followed by 15 consecutive 250-g batches of uninoculated celery, with each batch of diced product collected in a sterile Whirl-Pak bag. After dicing, all visually identifiable red pieces of Swiss chard were removed from each bag of diced product as well as from the dicer equipment, using sterile tweezers, and were subsequently weighed to quantify the spread of inoculated Swiss chard through the batches of diced celery. Samples of initially uninoculated celery without Swiss chard were then examined for numbers of *L. monocytogenes* as detailed below.

**L. monocytogenes growth on diced celery.** Inoculated celery sticks (600 g) were mechanically diced using the same dicer, with the diced celery collected in a sterile Whirl-Pak bag. Thereafter, equal portions were weighed into three sterile Whirl-Pak bags, which were subsequently closed and stored aerobically for 7 days at 4, 7, or 10°C for daily enumeration of *L. monocytogenes*. Generation times were only determined for samples stored at 10°C that exhibited significant growth. Values were obtained from the “best fit” line during logarithmic growth according to:

\[
G = \frac{t}{3.3 \log b/B}
\]

where *t* is the time in days, *b* is the *L. monocytogenes* population at time 1, and *B* is the *L. monocytogenes* population at time 2.

**Microbiological analysis.** All 25-g samples of diced celery were added to Whirl-Pak filter bags, diluted 1:4 in sterile 0.1% phosphate-buffered solution (PBS), and homogenized in a stomacher (Stomacher 400 Circulator, Seward, Worthington, UK) at 260 rpm for 1 min. The sample homogenates were then either appropriately diluted in sterile PBS and surface plated on modified Oxford agar (Neogen Corporation, Lansing, MI) or were processed by filtration using 0.45-µm-pore-size membrane filters (Millipore Corp., Billerica, MA) (lower detection limit of 0.4 CFU/g), which were placed on 60-mm-diameter petri plates containing modified Oxford agar. All colonies resembling *L. monocytogenes* were counted after 48 h of incubation at 37°C; no black colonies were observed in the control samples. Internalization of *Listeria* was a nonissue because preliminary studies showed that statistically similar numbers were recovered from both stomached and mechanically blended samples.

**Statistical analysis.** *L. monocytogenes* counts from triplicate experiments were converted to log CFU per gram and subjected to
L. monocytogenes growth in 15 batches of diced celery during storage. Celery stored at 4 and 7°C for 7 days yielded average Listeria population increases of 0.7 and 1.6 log CFU/g, respectively (Fig. 2A and 2B). At 4 and 7°C significant growth (P < 0.05) of Listeria was seen in a total of only 2 and 11 batches of previously uninoculated celery, respectively, whereas all 15 previously uninoculated batches yielded significantly (P < 0.05) greater average population increases of 3.4 log CFU/g after 7 days of storage at 10°C (Fig. 2C). Population increases at 10°C ranged from 2.6 to 4.2 log CFU/g, which occurred in batches 1 and 9, respectively.

Transfer of Swiss chard during dicing. Immersing the red Swiss chard stems in the five-strain L. monocytogenes cocktail (7.9 ± 0.1 log CFU/ml) yielded a population of 6.8 ± 0.4 log CFU/g after air drying, with the numbers of listeriae on Swiss chard remaining relatively unchanged at 6.6 ± 0.7 log CFU/g after initial dicing. After dicing the uninoculated celery and removing all visible pieces of Swiss chard, L. monocytogenes spread to all 15 batches of previously uninoculated celery (Fig. 1B). Similar (P > 0.05) populations of 4.7 ± 0.3 and 3.9 ± 0.2 log CFU/g were recovered from the first two batches of uninoculated celery, with batches 4 through 15 yielding a significantly lower (P < 0.05) average Listeria population of 1.7 log CFU/g.

After dicing 250-g batches of celery and the surrogate Swiss chard, followed by 15 250-g batches of celery, all visible pieces of Swiss chard were retrieved from each batch of celery and weighed. The first batch of celery diced following Swiss chard retained 25.0% ± 1.8% (62.5 g) of the surrogate, significantly more (P < 0.05) than that retrieved from the remaining batches of celery (Table 1). The percentages of Swiss chard spread to batches 2 to 15 were similar (P > 0.05), with each batch containing an average of 0.25% (0.6 g) of the surrogate. After dicing all 15 batches of celery, 4.33% (10.8 g) of Swiss chard remained on the equipment.

L. monocytogenes growth on inoculated diced celery. Celery stalks were inoculated with a five-strain L. monocytogenes suspension of 7.2 ± 0.2 log CFU/ml to obtain 3.2 ± 0.3 log CFU/g; then they were air dried and diced. During storage at 4, 7, and 10°C, L. monocytogenes reached maximum populations of 3.3 ± 0.6, 3.8 ± 0.3, and 5.2 ± 0.7 log CFU/g, respectively (Fig. 3). Populations of L. monocytogenes remained relatively unchanged in diced celery during storage at 4 and 7°C. However, significant growth (P < 0.05) was observed at 10°C after 7 days of storage, with a generation time of 0.87 days.

RESULTS

Transfer of L. monocytogenes from inoculated celery. Celery stalks were inoculated with a five-strain L. monocytogenes cocktail (7.8 ± 0.1 log CFU/ml) to obtain 5.6 ± 0.1 log CFU/g after air drying. After one uninoculated batch of celery was diced to prime the system, followed by the inoculated celery, L. monocytogenes populations on inoculated celery were statistically unchanged at 5.5 ± 0.2 log CFU/g immediately after dicing. Similar (P > 0.05) populations of 5.2 ± 0.1 log CFU/g were observed in the first 15 subsequently diced batches of uninoculated celery, with significantly (P < 0.05) lower populations seen in batches 2 and 3 (Fig. 1A). L. monocytogenes was recovered from all 15 batches of previously uncontaminated celery, reaching similar (P > 0.05) populations of 2.0 log CFU/g in batches 8 through 15.
from an inoculated mechanical slicer (8 log CFU/cm²) to delicatessen meat (40), both pathogens were recovered from all fractions of previously uninoculated product after processing.

One goal of the present study was to similarly quantify L. monocytogenes transfer and spread during mechanical dicing of celery. Dip inoculation was used to mimic field contamination from furrow irrigation or unintended flooding. Thus far, only retail surveys have been conducted for Listeria in celery, and all samples have been negative for the pathogen (12, 20, 35). Consequently, if contaminated prior to processing, celery would likely harbor very low numbers of Listeria due to its smooth surface structure. Thus, although it is far above expected levels of contamination, an inoculation level of 5.6 ± 0.1 log CFU/g was needed to obtain quantitative data at the lower limit of detection of 0.4 log CFU/g because pathogen recovery was expected to decline significantly with each subsequent batch of uninoculated celery. One study by Flores and Tamplin (16) found that the amount of final product contaminated was proportional to the initial inoculation level, whereas Vorst et al. (40) and Buchholz et al. (6) demonstrated that cross-contamination to uninoculated product became far more random when low inoculation levels (10^3 to 10^2 log CFU/cm²) were used. Although the extent of transfer observed in this study is unlikely in the fresh-cut industry, one outbreak of listeriosis was nonetheless traced to commercially diced celery as previously discussed (17, 18, 38). Hence, these transfer data, when modeled, will be useful in refining various risk assessments for diced produce.

Bacterial transfer during food processing has been shown to equilibrate over time. In the present study, Listeria

FIGURE 2. Populations of L. monocytogenes transferred from inoculated celery sticks (5.6 ± 0.1 log CFU/g) to uninoculated celery during mechanical dicing and subsequent storage at (A) 4°C, (B) 7°C, and (C) 10°C. Data reported are means ± standard deviations for three replicates.
TABLE 1. Percentage of Swiss chard recovered from diced celery after mechanical dicing of Swiss chard stems followed by celery

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean ± SD Swiss chard recovered (%)&lt;sup&gt;a,b&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>Initial Swiss chard</td>
<td>58.20 ± 7.55&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Celery</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>25.01 ± 1.81&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>1.50 ± 0.50&lt;sup&gt;c,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>0.97 ± 0.76&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>0.28 ± 0.19&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>0.14 ± 0.07&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>0.11 ± 0.07&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>0.06 ± 0.10&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>8</td>
<td>0.05 ± 0.05&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>9</td>
<td>0.06 ± 0.09&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>10</td>
<td>0.17 ± 0.29&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>11</td>
<td>0.03 ± 0.05&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>12</td>
<td>0.01 ± 0.00&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>13</td>
<td>0.02 ± 0.01&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>14</td>
<td>0.01 ± 0.02&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>15</td>
<td>0.06 ± 0.08&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Remaining on equipment after dicing</td>
<td>4.33 ± 1.89</td>
</tr>
<tr>
<td>Unaccounted for</td>
<td>8.97 ± 12.19</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean percentages ± standard deviations are based on the total weight of Swiss chard (250 g) that was initially diced (n = 3).

<sup>b</sup> Means followed by different letters represent the percentages of Swiss chard recovered that differed significantly (P < 0.05) between samples.

Swiss chard was retrieved from all 15 batches of uninoculated celery after dicing. The highest levels of Swiss chard were seen in the first batch of uninoculated celery, with steady state attained thereafter (Table 1). A correlation between the amount of contaminated product in the system and the level of contamination achieved is suggested by the similar transfer patterns observed during the initial study with inoculated celery, and after quantification of *Listeria* in celery samples free of inoculated Swiss chard (Fig. 1A and 1B). Initial decreases in populations were observed over the first three batches, and steady populations were maintained after batches 8 and 4, respectively. In another study that used radicchio as a surrogate for inoculated lettuce during leafy greens processing, Buchholz et al. (7) also found that the decrease in bacterial transfer to uninoculated lettuce coincided with the decreased amount of inoculated product in the system. Whereas most of the contaminant Swiss chard was removed from the system by the end of dicing, the remaining 4.3% of the surrogate on the equipment would be expected to spread to additional celery over time.

Foods posing the highest risk of listeriosis are those that become contaminated during handling and that support growth of the pathogen to potentially hazardous levels during subsequent storage (36). Of particular concern with *L. monocytogenes* is its ability to multiply at temperatures as low as 0°C (37). Several studies have assessed temperature abuse during consumer handling with temperatures ranging from 0.9 to 11.4°C (mean of 7.4°C) during storage of ready-to-eat salads (22, 24). Because temperature abuse has been a factor in listeriosis outbreaks linked to coleslaw (31), chocolate milk (11), and rice salad (30), growth potential for *L. monocytogenes* in diced celery containing 3.2 log CFU/g was assessed over a range of storage temperatures. The 2.4 log CFU/g difference in inoculation levels between the transfer and storage studies after dip inoculating celery in a *L. monocytogenes* suspension containing ~7 log CFU/ml may be due to seasonal and/or varietal differences associated with the exterior surface of the celery, which...
could alter bacterial attachment during inoculation and subsequent drying.

Past studies have demonstrated a lack of significant \( L.\) *monocytogenes* growth on shredded and whole leaf lettuce (3) and cooked sweet corn and bean sprouts (34) when stored at 5°C for 8 days and 3°C for 10 days, respectively. However, under abusive conditions \( L.\) *monocytogenes* reportedly grew on whole and cut lettuce during 10 days of storage at 10°C (3). In the present study, \( L.\) *monocytogenes* behaved similarly on diced celery. The amount of growth achieved by \( L.\) *monocytogenes* on diced celery decreased with increasing storage temperature in both the transfer and growth studies. The impact of storage temperature on pathogen levels was addressed in both studies because the latter allowed more frequent sampling and a uniform initial inoculation, as opposed to the former, in which the starting level continually decreased during dicing. In contrast to our findings, Prakash et al. (29) found that \( L.\) *monocytogenes* populations decreased \( \sim 0.3 \) log CFU/g on diced celery during 7 days of storage at 5°C. According to Vandamm et al. (39), during 7 days of storage \( L.\) *monocytogenes* populations on fresh-cut celery decreased by \( \sim 1 \) log CFU/g at 4°C and increased by 0.5 log CFU/g at 12°C.

These discrepancies in \( L.\) *monocytogenes* behavior can likely be attributed to the use of different \( L.\) *monocytogenes* strains, the method of inoculation (spot versus dip inoculation), the state of the celery upon arrival, the method of preparation, and differences in the celery surface-to-weight ratio (3). In our preliminary work (data not shown), \( L.\) *monocytogenes* populations were up to 28% higher on the cut ends of dip-inoculated celery sticks as compared with the smooth, relatively dry, uncut surfaces, suggesting that \( L.\) *monocytogenes* preferentially attaches to cut or otherwise damaged sites. In the two previous studies (29, 39), celery was purchased as a ready-to-eat, fresh-cut product that had presumably been commercially washed with a chemical sanitizer before packaging. In addition, the cut celery pieces used by Vandamm et al. (39) were far larger than the diced pieces generated in the present study. Hence, the larger surface-to-volume ratio of our diced celery likely increased water and nutrient availability for pathogen growth (18). Microbial growth is also partially dictated by the extent of damage at the cut surface of fresh produce. According to Gleeson and O’Beirne (18), greater growth of \( L.\) *innocua* was observed when fresh-cut leafy greens were heavily damaged as a result of using dull as opposed to sharp blades. The celery used in our study was similarly subjected to damage during mechanical dicing, as opposed to hand dicing used in the study by Vandamm et al. (39).

In summary, this study demonstrates that pathogens from celery contaminated with \( L.\) *monocytogenes* can transfer to subsequently processed product. In addition, \( L.\) *monocytogenes* was able to reach potentially hazardous levels on diced celery during storage. Hence, in addition to mitigation strategies during dicing, the final product needs to be properly refrigerated to minimize potential consumer health risks.

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