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Transfer and Inactivation of *Listeria monocytogenes* during Pilot-Scale Dicing and Flume Washing of Onions

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Abstract: This study assessed the extent of *L. monocytogenes* transfer from onions to the surface of a commercial dicer, from inoculated onions to uninoculated onions, and the efficacy of various sanitizers during the subsequent flume washing of diced onions. Spanish yellow onions (*Allium cepa* L.) were dip-inoculated in a 3-strain avirulent *L. monocytogenes* cocktail (5.9 or 4.2 log CFU/50 g) and air-dried. After dicing one 2.2 kg batch of onions inoculated at ~5.9 log CFU/50 g followed by ten uninoculated batches of 2.2 kg each, *L. monocytogenes* progressively decreased from 4.6 to 2.6 log CFU/50 g in baches 1 through 10, respectively. After onions inoculated at ~4.0 log CFU/g were diced and flume washed for 2 min in tap water, electrolyzed water containing 55 ppm free chlorine, 80 ppm free chlorine from a commercial sanitizer, or 80 ppm peroxyacetic acid and dewatered on a mechanical shaker table, *L. monocytogenes* populations decreased 0.4, 0.3, 1.4, and 1.0 log, respectively, with populations of ~1.2 log CFU/mL in water for all three sanitizers. These findings should be useful in future risk assessments and aid in the development of improved industry guidelines to better enhance the safety of diced onions.

Keywords: *Listeria monocytogenes*; onions; cross-contamination; dicing; chlorine; electrolyzed water; peroxyacetic acid

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1. Introduction

Increased consumption of raw, minimally processed, fresh-cut produce is raising many new safety concerns regarding the risk of foodborne illness. After two major recalls in 2007 and 2012, one of which involved ~22,000 kg of diced onions from a single producer, the safety of raw onions continues to be under scrutiny [1,2]. Due to the widespread use of onions as a food ingredient, the 2012 recall led to 11 additional recalls issued across the United States and Canada, all without incident [2]. In follow-up investigations, the implicated strain of *L. monocytogenes* was recovered from multiple sites within the processing facility, including a chute under an inspection table, a shroud on a peeling machine, and the loading hopper of a peeling machine [3], indicating the pathogen likely entered the facility on the unpeeled onions and then spread to subsequent onions during processing. Interestingly, in 2023, 80 cases of salmonellosis across 23 states were traced to diced onions from this same commercial producer with the outbreak strain also recovered from environmental samples where the onions were grown [4]. While these reports emphasize the potential spread of pathogens during commercial dicing, in other instances, *Listeria*-contaminated salads containing onions as an ingredient could not be linked to onions as the source of the contamination [5–7].

The incidence of *Listeria* in fresh produce is generally considered to be very low, with multiple studies failing to detect *Listeria* in whole intact onions at retail [8,9]. However, in 2022, whole Vidalia onions contaminated with *L. monocytogenes* were recalled by one Georgia grower from six states without incident [10]. Numerous studies have identified and tracked the presence of *Listeria* and other pathogens in produce packinghouses as well as on product contact surfaces during the processing of fresh-cut produce [11–17].

Commercial onion processing begins with "topping and tailing" where the root and sprout ends of the onion bulb are simultaneously removed by blades either before or after the outer skin is peeled using a corkscrew-type conveyer. Thereafter, the onions are mechanically diced, flume washed in water containing 50 to 200 ppm chlorine, and then mechanically dewatered for packaging. However, due to the potential spread of *Listeria* during onion dicing and the limited efficacy of sanitizers, commercially prepared diced onions need to be properly refrigerated to minimize the risk of growth and adverse public health consequences [18,19].

A series of studies have been published on the transfer of *L. monocytogenes* as well as *Escherichia coli* O157:H7 and *Salmonella* during the shredding of leafy greens [20,21], dicing of celery [22], and slicing/dicing of tomatoes [23–26]. In several reports, log-linear, Weibull, and exponential decay models [27–30] were used to better describe the trends in bacterial transfer for different processing scenarios.

In a follow up to our previous work demonstrating the spread of *L. monocytogenes* from one inoculated to twenty uninoculated onions during mechanical slicing [24], this study aimed to assess (1) the ability of *L. monocytogenes* to transfer from inoculated onions to the components of an industry-scale mechanical dicer and then to sequentially diced uninoculated onions and (2) the efficacy of several commonly used commercial sanitizers during the flume washing of *Listeria*-inoculated diced onions.

2. Materials and Methods

2.1. Experimental Design

Transfer of three avirulent *L. monocytogenes* strains (M3, J22F, J29H) from 2.2 kg of whole-surface-inoculated onions to the components of a commercial dicer was assessed. Subsequently, *L. monocytogenes* transfer from 2.2 kg of surface-inoculated onions to ten 2.2 kg batches of uninoculated onions during mechanical dicing was quantified by direct plating, with these findings then subjected to mathematical modeling to predict the extent of transfer. Finally, *Listeria*-inoculated diced onions were used to assess the efficacy of flume washing in water alone, and in water containing 55 ppm free chlorine, 80 ppm free chlorine, or 80 ppm peroxyacetic acid.

2.2. L. monocytogenes Strains

Three avirulent *L. monocytogenes* strains—M3 serotype 1/2a (parent was Mackaness strain), J22F serotype 4b (parent was NCTC 10527), and J29H serotype 4b (parent was NCTC 10527) (obtained from Dr. Sophia Kathariou, North Carolina State University, Raleigh, NC, USA) were used for all experiments. All cultures were stored at −80 °C in trypticase soy broth containing 0.6% (*w*/*v*) yeast extract (TSBYE, Becton, Dickinson and Company, Sparks, MD, USA) and 10% (*v*/*v*) glycerol. These strains were streaked for isolation to plates of trypticase soy agar containing 0.6% (*w*/*v*) yeast extract (TSAYE, Becton, Dickinson and Company) and incubated 24 h at 35 °C. Thereafter, an isolated colony of each strain underwent two consecutive 24 h/35 °C transfers in TSBYE. A cocktail was prepared by combining equal volumes of the three avirulent strains, followed by appropriate dilution to obtain populations of ~7.0 or 5.5 log CFU/mL for onion inoculation, with these levels confirmed by surface plating on Modified Oxford Agar (MOX, Neogen Corp., Lansing, MI, USA).

2.3. Onions

Spanish yellow onions (*Allium cepa* L.) were purchased from a local supplier (Stan Setas Produce Company, Lansing, MI, USA) and stored at 4 °C for no more than 7 d before use. The root and sprout portions of each whole onion were removed using a sterile knife. After hand peeling the outer skin, the onions were tempered to room temperature (23 °C ± 2 °C) and weighed prior to dicing.

2.4. Onion Dicer

A large-scale commercial dicer (Model H-A, Urschell, Valparaiso, IN, USA) located in the Department of Food Science and Human Nutrition Fruit and Vegetable Processing Laboratory (Michigan State University, East Lansing, MI, USA) was used for all experiments (Figure 1). Eight 100 cm^2 product contact areas of the dicer—the side and bottom of the loading drum, front and back of the rotating paddles, slicing blade, cross-cut blade, dicing blade, and the discharge chute were chosen for sampling.

2.5. L. monocytogenes Transfer during Dicing

Whole peeled onions were immersed in the diluted 3-strain avirulent cocktail containing ~7.0 or 5.5 log CFU/mL for 2 min and then air-dried in a biosafety cabinet for 90 min, giving initial populations of ~5.9, or 4.2 log CFU/50 g. Transfer of *L. monocytogenes* to the commercial dicer was assessed by dicing one 2.2 kg batch of inoculated onions. Eight 100 cm^2 areas of the dicer were sampled after disassembly using the 1-ply composite tissue method [31]. One 50 g diced onion sample was collected and assessed for numbers of *Listeria* to confirm the initial inoculation level.

(**A**)

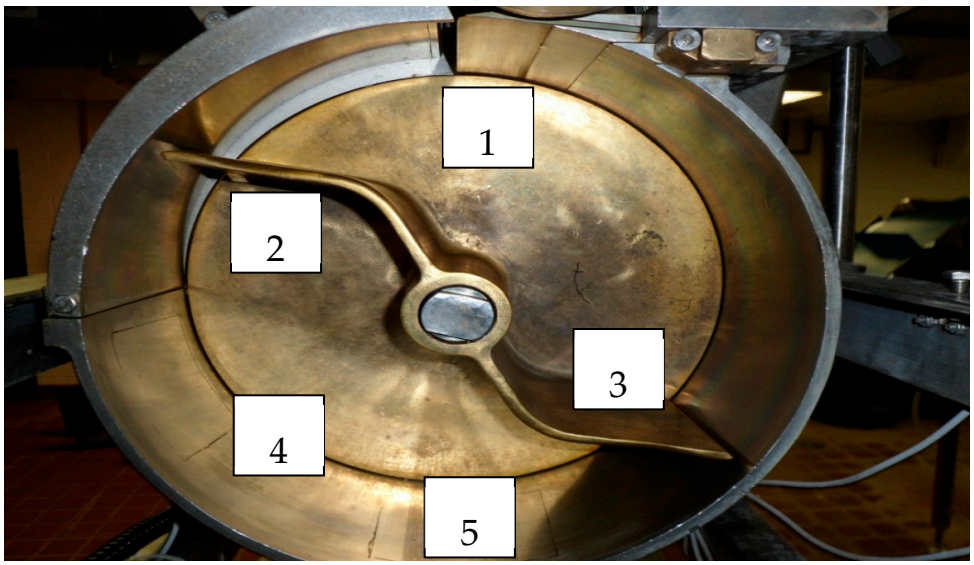

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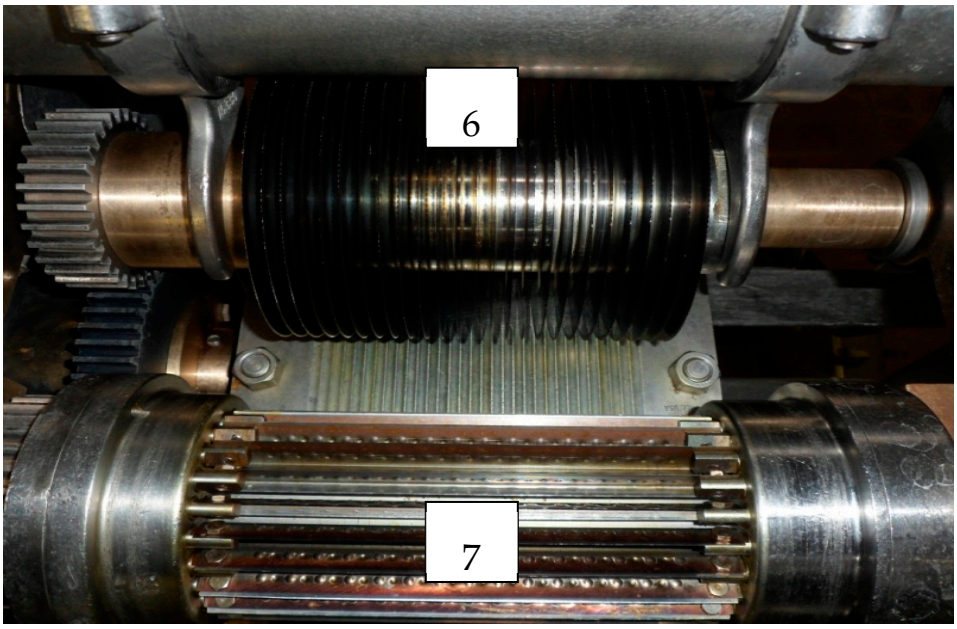
Figure 1. *Cont.*



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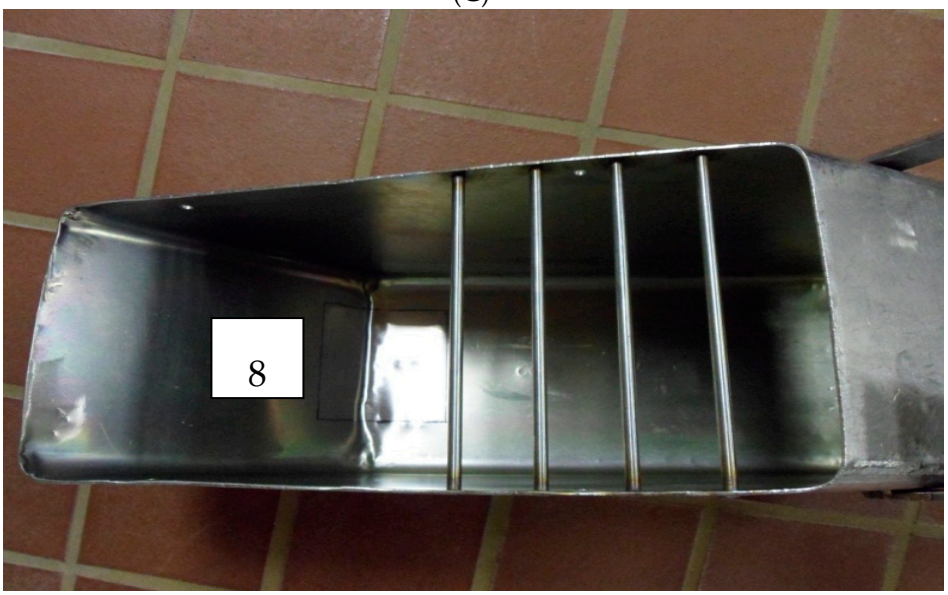


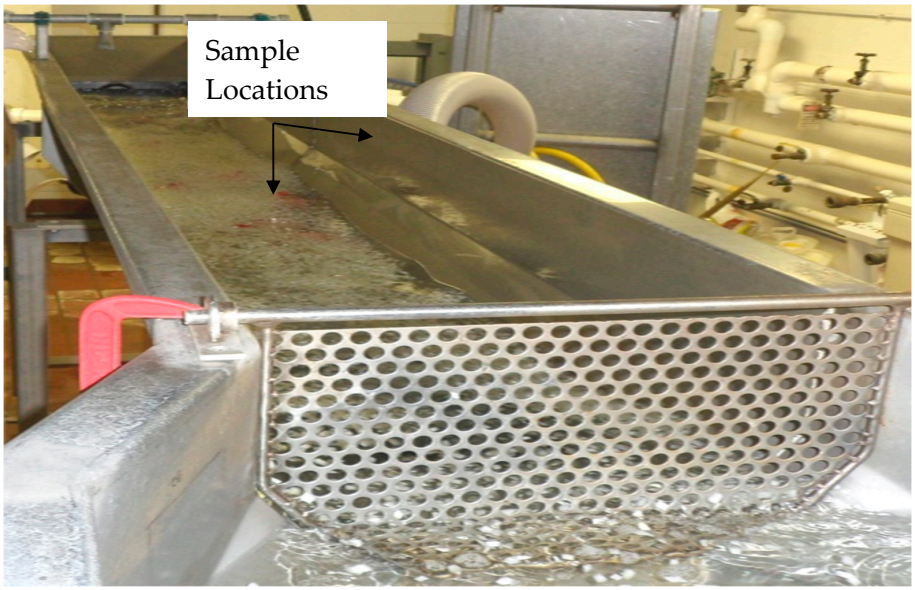
(**D**)

Figure 1. (**A**) Commercial dicer. (**B**) Commercial dicer sampling locations: slicer blade (1), paddles (2, 3), and drum (4, 5). (**C**) Commercial dicer sampling locations: rolling dicer blades (6) and cross-cut blades (7). (**D**) Commercial dicer sampling location: chute (8).

Transfer of *L. monocytogenes* was also assessed by dicing one 2.2 kg batch of inoculated onions immediately followed by ten 2.2 kg batches of uninoculated onions. Each of the 11 diced onion batches was collected separately and mixed by hand with one 50 g sample collected at random for quantification of *Listeria*. After dicing the 10 batches of uninoculated onions, the dicer was disassembled and the same eight 100 cm^2 areas were again sampled using the 1-ply composite tissue method. All dicing experiments were conducted in triplicate. After each experiment, the dicer was disassembled, washed with a brush to physically remove debris, and then sanitized with 200 ppm Quorum V (Ecolab, Saint Paul, MN, USA), rinsed with tap water and allowed to dry before reassembly.

2.6. Flume Washing with Various Sanitizers

The processing equipment for washing the diced onions was also located in the Department of Food Science and Human Nutrition Fruit and Vegetable Processing Laboratory. This processing line included a water recirculation tank (~1000-L capacity), 3.6 m-long flume tank (~130-L capacity; Heinzen Manufacturing Inc., Gilroy, CA, USA) and a dewatering shaker table operated by a 1-horsepower washdown duty motor (Baldor Electric Co., Ft. Smith, AR, USA) at 1760 rpm. The water recirculation tank containing 700 L of tap water (~15 °C) with or without a sanitizer was connected by a rigid plastic discharge hose (4.5 m × 0.1 m) to the flume tank by a centrifugal pump (model XB754FHA, Sterling Electric, Inc., Irvine, CA, USA). A custom-made stainless-steel screen was attached to the end of flume tank to retain the diced onions for 2 min of washing (Figure 2). All washing experiments were conducted in triplicate. After each experiment, the disassembled dicer and flume line were rinsed to physically remove debris, and then sanitized with 200 ppm Quorum V (Ecolab, Saint Paul, MN, USA), rinsed with tap water and allowed to air dry.



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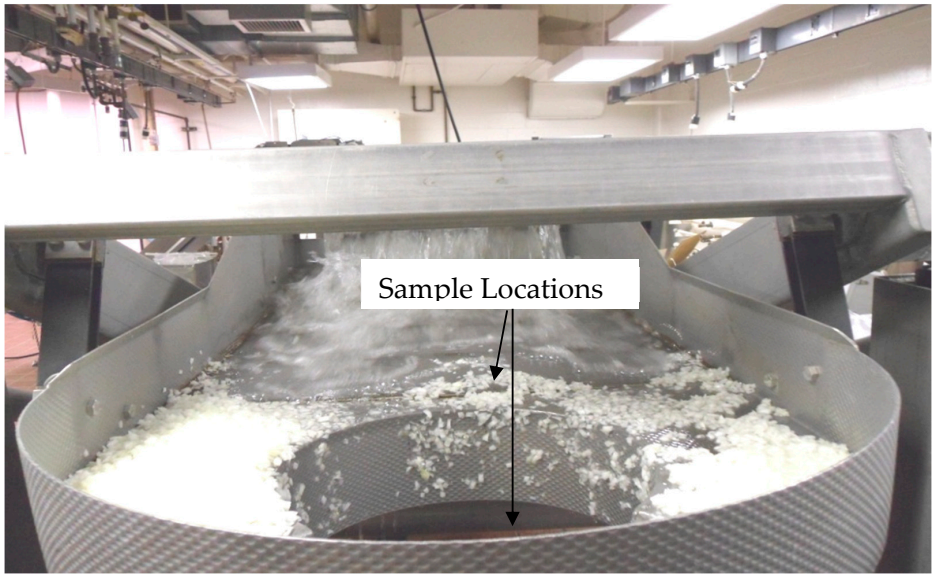


(**B**)

Figure 2. Processing equipment: (**A**) 3.6 m flume tank and (**B**) dewatering shaker table.

2.7. Sanitizers

Three different sanitizer treatments were used in this study. A commercial chlorine-based sanitizer (XY-12, Ecolab, St. Paul, MN, USA) was diluted in tap water to contain 80 ppm free chlorine and adjusted to pH of 6.5 with 10% (*w*/*v*) citric acid (CA). Electrolyzed water containing ~55 ppm free chlorine was produced on site using a commercial generator (PathoSans®, Spraying Systems Co., Westfield, IN, USA). A peroxyacetic acid-based sanitizer (Tsunami-100, Ecolab, St. Paul, MN, USA) was diluted in tap water to contain to 80 ppm peroxyacetic acid (PAA). Chlorine test kit 321 (Ecolab) was used to measure chlorine levels, and peroxyacetic acid test kit 311 (Ecolab) was used to measure PAA concentrations.

2.8. Evaluation of Sanitizer Efficacy

Whole peeled onions (9.1 kg) were immersed in the diluted 3-strain avirulent cocktail containing ~7.0 log CFU/mL for 2 min and then air-dried in a biosafety cabinet for 90 min, giving a population of ~4.0 log CFU/g. After dicing the 9.1 kg of inoculated onions with the same commercial dicer, seven 50 g samples were weighed into mesh produce bags (pore size < 0.5 cm), which were then placed in the flume tank containing 90 L of sanitizer-free water, 80 ppm free chlorine, 80 ppm PAA, or 55 ppm free chlorine as electrolyzed water, and vigorously agitated by hand for 2 min. During sanitizer exposure, 50 mL water and 50 g onion samples were collected every 20 s. After 2 min of washing, the flume pump was activated, and the screen was raised to flush the diced onions across the shaker table with a final 50 mL water and 50 g onion sample collected after 20 s of dewatering. After onion and water sample collection, the flume tank was emptied, and two surface samples (100 cm^2) were taken from the flume tank and the shaker table as shown in Figure 2.

2.9. Microbial Analysis

All samples from the *Listeria* transfer experiments were added to Whirl-pak® bags containing University of Vermont Medium (UVM, Neogen), homogenized by stomaching (Stomacher 400 Circulator, Seward USA, Davie, FL, USA) for 1 min at 300 rpm, appropriately diluted in phosphate-buffered solution (PBS), and plated, with or without prior filtration through a 0.45 µm filter membrane using a vacuum pump (Model E46046, EMD Millipore Corporation, Billerica, MA, USA), on MOX with the UVM-diluted samples enriched at 35 °C. All plates were examined for typical *Listeria* colonies after 48 h of incubation at 35 °C. When samples were negative for *Listeria* by direct plating, the UVM enrichments were streaked to plates of MOX, incubated at 35 °C for 48 h, and then examined for the presence or absence of *Listeria*. All samples from the sanitizer efficacy studies were added to Whirl-pak® bags containing Neutralizing Buffer (Difco, BD, Franklin Lakes, NJ, USA), homogenized by stomaching for 1 min at 300 rpm, appropriately diluted in PBS, and plated, with or without prior filtration through a 0.45 µm filter membrane on MOX to quantify *Listeria*.

2.10. Statistical Analysis

All experiments were performed in triplicate. *Listeria* populations were reported as log CFU/onion ± SE for the inoculated and uninoculated onions or log CFU/100 cm^2 ± SE for the dicer surface samples. For subsequent analysis of the *Listeria* transfer data, samples positive by enrichment were assumed to contain 1 CFU, whereas surface samples negative by enrichment were assumed to contain 0.5 CFU. Onion samples negative by enrichment were not included in the analysis. The Tukey–Kramer Honestly Significant Difference Test was performed using JMP 10 (SAS Institute Inc., Cary, NC, USA). Statistical significance was set at $p < 0.05$.

2.11. Evaluation of Transfer Model

An exponential decay model from a previous study (Sheen and Hwang, 2010) was used to describe the *L. monocytogenes* transfer pattern during dicing of onions. The model used to fit the data is shown in Equation (1):

$$Y = A \cdot e^{X/B} \quad (1)$$

where Y (dependent variable) is the log CFU/onion transferred and X (independent variable) is the number of the specific uninoculated batch of onions that was diced. A and B are the transfer model parameters. The above equation was fitted using the *nlinfit* algorithm of MATLAB (R2012a, MathWorks, Natick, MA, USA). The estimated parameters, normalized root mean squared errors (NRMSE) of the model, shown in Equation (2), and asymptotic 95% confidence intervals of the parameters were then estimated as follows:

$$\text{NRMSE} = \frac{\text{RMSE}}{(\text{Ymax} - \text{Ymin})} \quad (2)$$

where NRMSE is the normalized root mean squared error, RMSE is the root mean squared error, Ymax is the maximum value taken by Y, and Ymin is the minimum value taken by Y.

3. Results

3.1. Transfer from Inoculated Onion to Dicer

After dicing one batch of inoculated onions, *L. monocytogenes* was present on all dicer surfaces sampled with similar trends observed at both inoculation levels (Figure 3). The drum and slicer blade yielded average *Listeria* populations of 4.0 ± 0.2 and 4.2 ± 0.5, and 2.7 ± 0.4 and 3.6 ± 0.3 log CFU/100 cm^2 after dicing a single batch of onions inoculated to contain ~5.9 or 4.2 log CFU/50 g, respectively. *Listeria* contaminated the remaining surfaces at varying levels, with the paddles and cross-cut blades yielding lower levels than the other components.

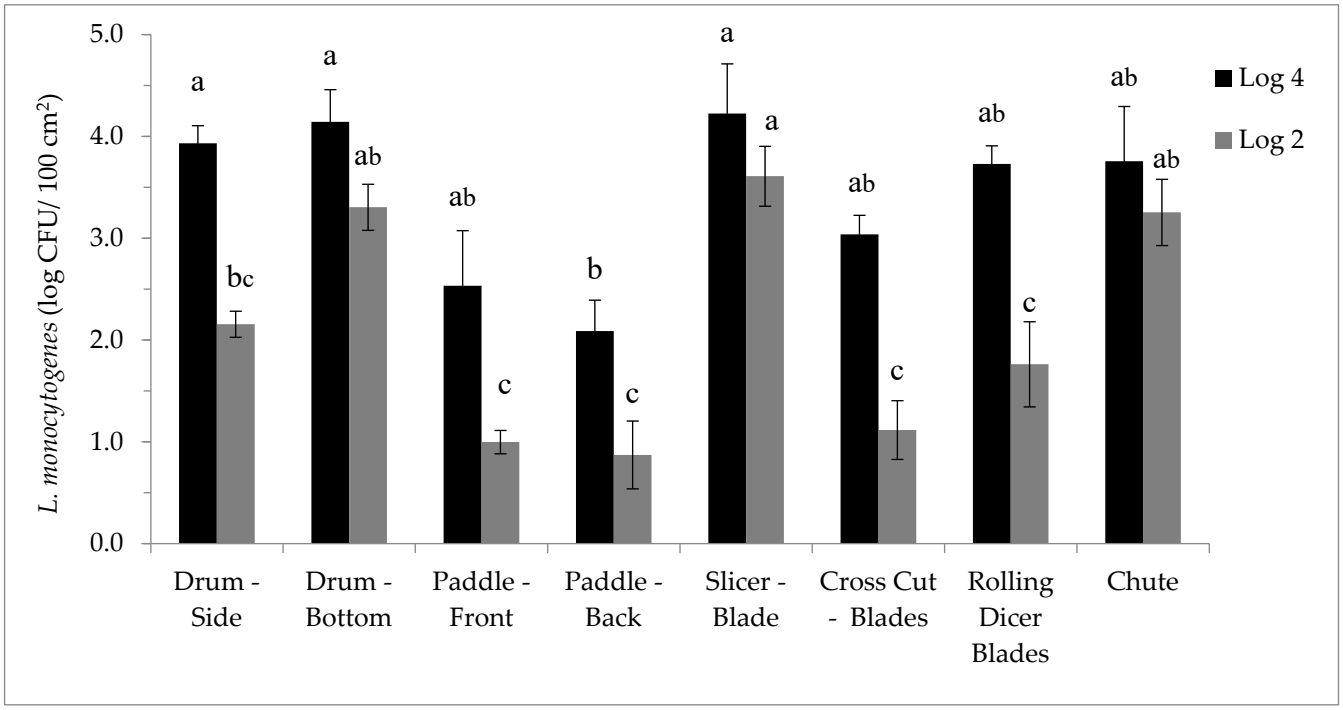


Figure 3. *L. monocytogenes* transfer from inoculated onions (5.9 or 4.2 log CFU/50 g) to different dicer components. Values were compared between components at the same inoculation level. Bars with different letters are significantly different.

At the lower inoculation level, there was no significant ($p > 0.05$) difference in the *Listeria* populations retained on the dicer components after dicing 10 batches of uninoculated onions (Figure 4). However, at the higher inoculation level, the drum bottom and circular dicing blades retained significantly ($p < 0.05$) higher populations of *L. monocytogenes* than the other components (Figure 5).

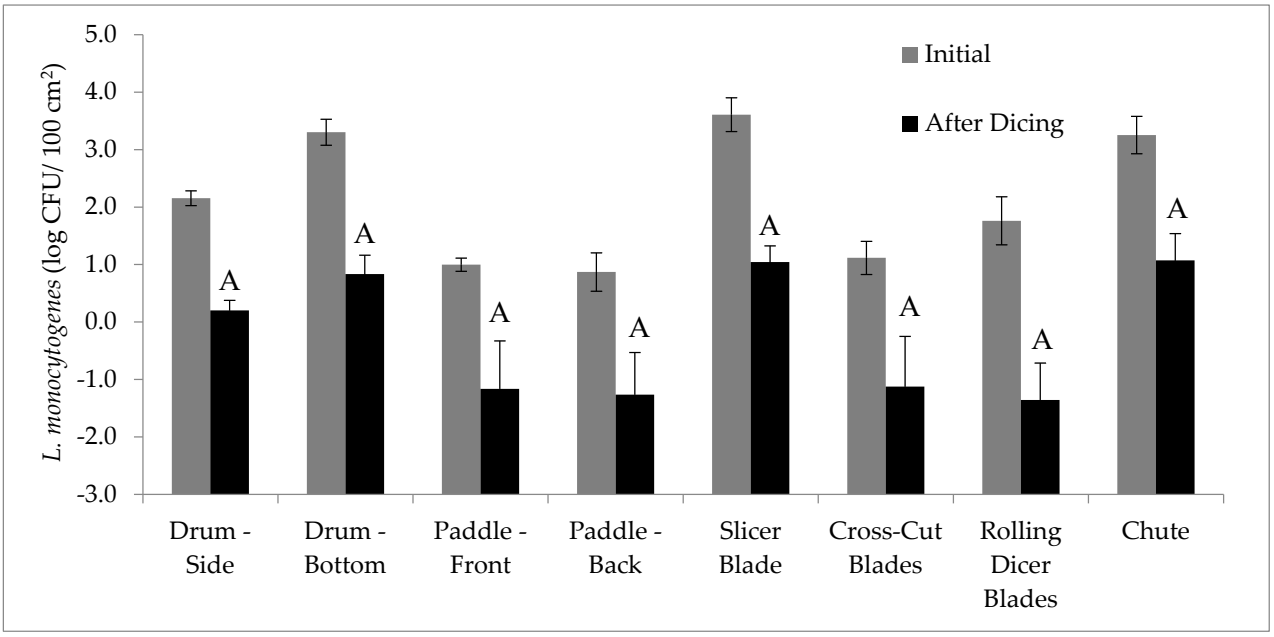


Figure 4. *Listeria* populations remaining on the different dicer surfaces after dicing one inoculated (4.2 log CFU/50 g) followed by 10 uninoculated batches of onions. Populations were compared across the different components after dicing with A denoting no significant difference.

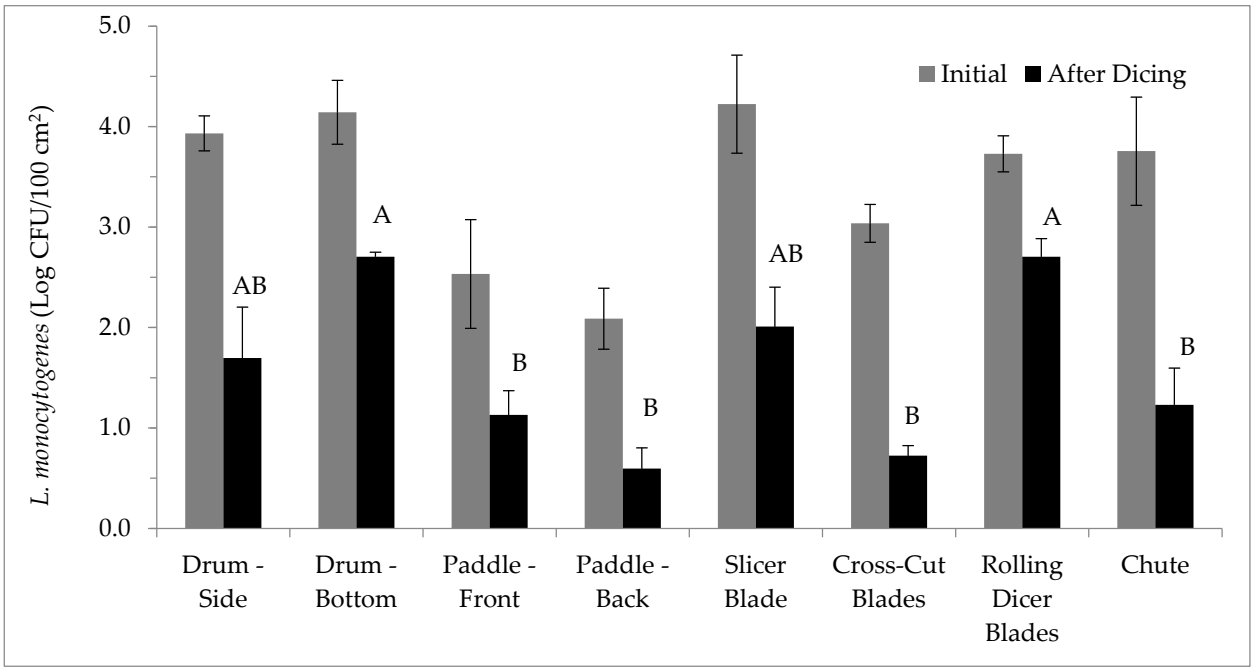
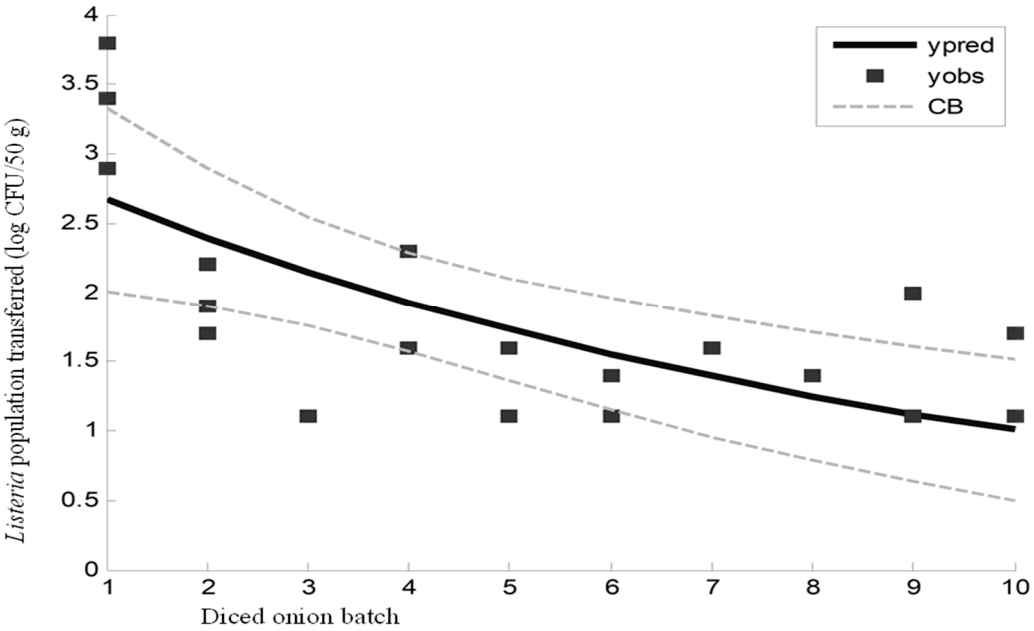


Figure 5. *Listeria* populations remaining on the different dicer surfaces after dicing one inoculated (5.9 log CFU/50 g) followed by 10 uninoculated batches of onions. After dicing bars with different letters are significantly different.

3.2. Transfer from Inoculated to Subsequently Diced Uninoculated Onions

After dicing one batch of inoculated onions followed by 10 batches of uninoculated onions, *Listeria* was detected in at least one of three replicates by enrichment at the low inoculation level. At the higher inoculation level of ~5.9 log CFU/50 g, *Listeria* populations in batches 1, 5, and 10 averaged 4.6 ± 0.1, 3.0 ± 0.1, and 2.3 ± 0.2 log CFU/50 g, respectively (Figure 6). The exponential model reasonably fit the lower and higher inoculation level transfer data with NRMSEs of 0.17 and 0.20, respectively (Table 1).



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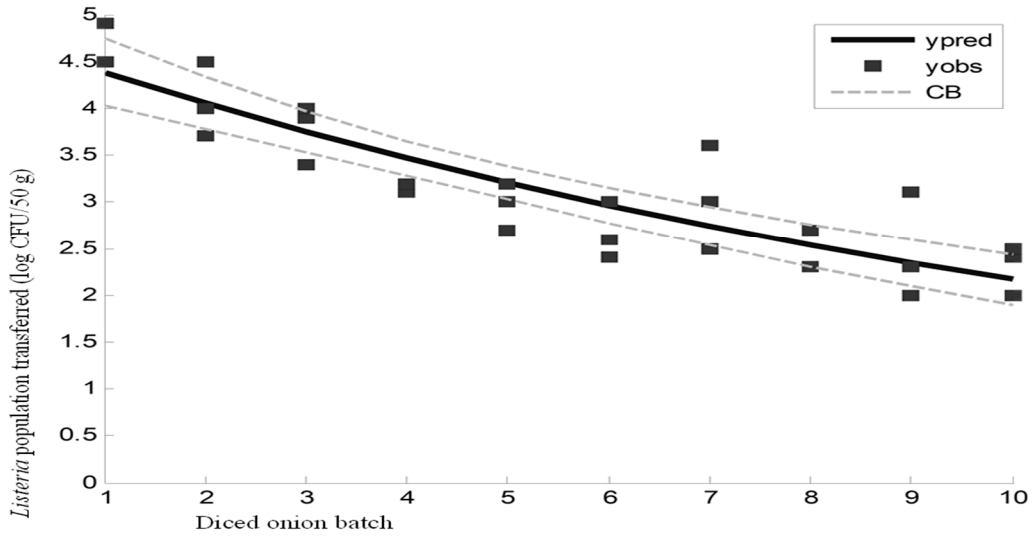


(**B**)

Figure 6. (**A**). Predicted *L. monocytogenes* transfer from one batch of inoculated onions batch (4.2 log CFU/50 g) to 10 batches of uninoculated onions. ypred is the prediction line; yobs is the observed line; CB is the confidence band for the prediction line. (**B**). Predicted *L. monocytogenes* transfer from one batch of inoculated onions batch (5.9 log CFU/50 g) to 10 batches of uninoculated onions. ypred is the prediction line; yobs is the observed line; CB is the confidence band for the prediction line.

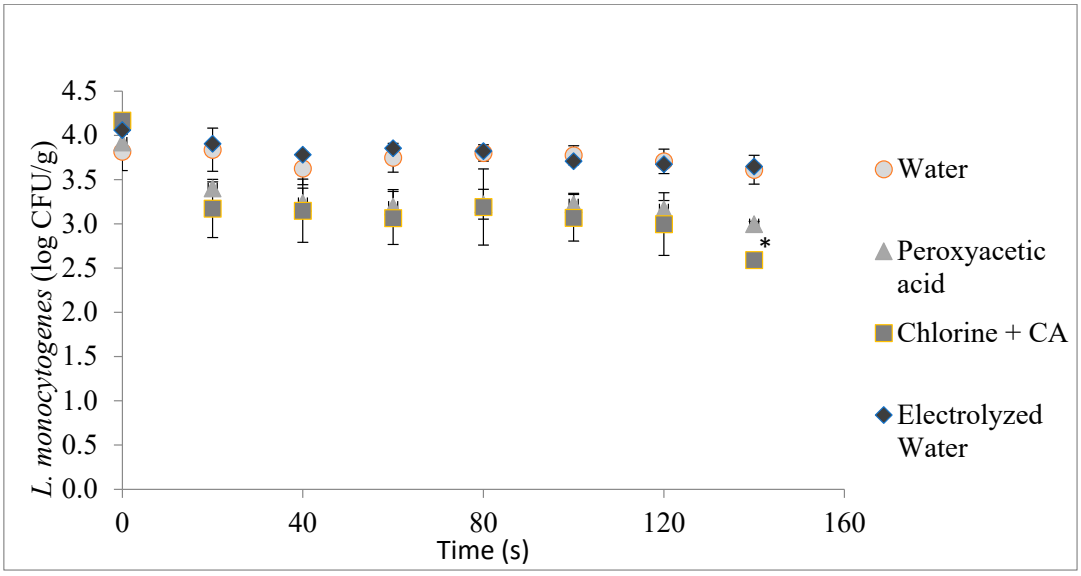
Table 1. Model parameters A and B for *L. monocytogenes* transfer from one inoculated onion to 10 uninoculated batches of onions during dicing.

Inoculation Level (log CFU/50 g)	A (95% CI) [a]	B (95% CI)	NRMSE [b] (log CFU/50 g)
4.2	2.98 (2.27, 3.68)	−9.23 (−13.96,−4.5)	0.17
5.9	4.74 (4.37,5.11)	−12.76 (−15.29,−10.23)	0.20

[a] Coefficient value (95% confidence interval). [b] Normalized root mean squared error for the transfer model.

3.3. Sanitizer Evaluation

Diced onions yielded *Listeria* populations of 3.6, 2.6, 3.0, and 3.7 log CFU/g after 2 min of washing in tap water, 80 ppm free chlorine, 80 ppm PAA, and 55 ppm free chlorine followed by shaker table dewatering. The chlorine-based sanitizer was significantly ($p < 0.05$) better than tap water, yielding a ~1.4 log CFU/g reduction (Figure 7A).



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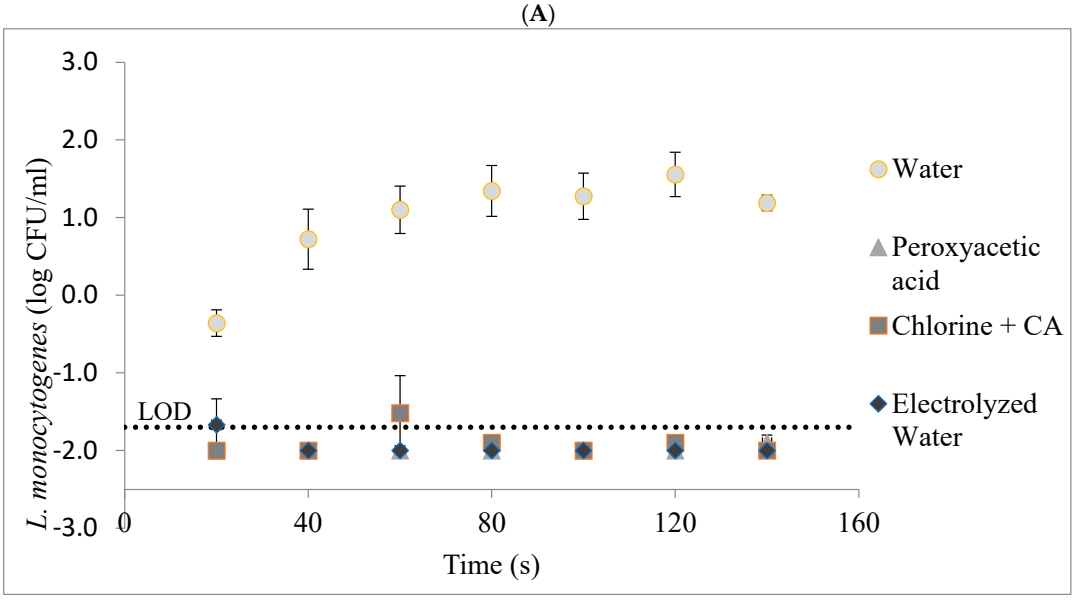

(**B**)

Figure 7. (**A**). *Listeria* populations on diced onions during washing and shaker table dewatering. * Denotes significant difference between treatment and water control. (**B**). *Listeria* populations in water during washing and shaker table dewatering of diced onion.

Listeria populations in sanitizer-free water increased to ~1.2 log CFU/mL during washing (Figure 7B). During the first minute of washing with a sanitizer, *Listeria* was quantifiable in three water samples at or near the limit of detection, which was attributed to the accumulation of onion particulates on the filter membrane during sample processing. Surface samples yielded significantly ($p < 0.05$) higher *Listeria* populations when sanitizers were not used (Table 2).

Table 2. *Listeria* populations (log CFU/100 cm^2) on the flume tank and shaker table after onion dicing and dewatering.

Equipment Surface	Water	Electrolyzed Water	Chlorine + Citric Acid	Peroxyacetic Acid
Flume Tank	1.6 ± 0.3^{a} * (6/6)	-0.3 ± 0.00^{b} (0/6)	-0.3 ± 0.0^{b} (0/6)	-0.1 ± 0.2^{b} (1/6)
Shaker Table	2.1 ± 0.3^{a} (6/6)	0.5 ± 0.3^{b} (4/6)	0.6 ± 0.2^{b} (5/6)	-0.3 ± 0.1^{b} (1/6)

* Fraction represents number of quantifiable samples. Populations on equipment surface were compared across sanitizers. Rows with different letters are significantly different.

4. Discussion

The *Listeria* transfer results during onion dicing are consistent with other studies showing the ability of foodborne pathogens to cross-contaminate large quantities of previously uncontaminated celery [22], leafy greens [32], and tomatoes [25,26] during dicing, slicing, and shredding. When 9.1.kg of radicchio was inoculated with *E. coli* O157:H7 at 6.0 log CFU/g, mechanically shredded, conveyed, flume washed in sanitizer-free water, and centrifugally dried, followed by 907 kg of uninoculated iceberg lettuce, *E. coli* O157:H7 was recovered from all of the processed lettuce indicating that even a small amount of contaminated product can have serious consequences [32]. When Kaminski, Davidson, and Ryser [22] mechanically hand-diced 250 g of celery inoculated with *L. monocytogenes* at 5.6 log CFU/g followed by 15 250-g batches of uninoculated celery, *Listeria* populations progressively decreased from 5.2 log CFU/g in batch 1 to 2.0 log CFU/g in batch 15. In the only other reported study using the same commercial dicer, Wang and Ryser [26] reported a 2.2 log reduction in *Salmonella* when one 0.9 kg batch of Roma tomatoes was diced followed by ten uninoculated batches compared to the 2.3 log reduction that was for *L. monocytogenes* in diced onions. Therefore, the extent to which *Salmonella* and *Listeria* spread appears to be unaffected by the type of product being diced or sliced as also shown by Alnughaymishi [33].

In our study, areas of the commercial dicer most prone to *Listeria* contamination after onion dicing included the rolling dicing blades, loading hopper, and slicer blade (Figure 3). Similarly, Buchholz, Davidson, Marks, Todd, and Ryser [32] showed that radicchio buildup was most evident on the cutting wheel and discharge chute of the shredder with statistically similar populations of *E. coli* O157:H7 recovered from the shredder, flume tank, and shaker table after processing 907 kg of iceberg lettuce. Kaminski, Davidson, and Ryser [22] also recovered 10.8 of 250 g of red Swiss chard from a mechanical hand dicer after dicing 15 250 g baches of celery with the retained Swiss chard expected to spread to additional product over time.

Proper cleaning protocols for both food and non-food contact surfaces in food processing are important to minimize the risk of product contamination by *Listeria*. Lundén, Autio, and Korkeala, ref. [34] showed that a mechanical dicing machine was the vehicle for a resident *Listeria* population that moved between three processing plants, with the dicer blades identified as one source of contamination. The 2012 recall prompted actions by the responsible party that included a cleaning and sanitizing protocol to eliminate *L. monocytogenes* from the facility. However, after actions were taken, the same strain was again identified on various equipment surfaces, demonstrating the difficulty in effectively removing colonized *Listeria* [1–3].

Sanitizers are clearly needed in flume water to minimize cross-contamination from the water during washing [35]. This study supports previous work showing that chlorine-based sanitizers are effective when the organic load is low [36–39]. Sanitizers are far less effective against Listeria and other bacterial pathogens when the product is contaminated. While the presence of *Listeria* on diced onions after flume washing raises additional concerns regarding potential migration into the product through cut surfaces, this study did not evaluate the location of *Listeria* on or in diced onion after dicing. Hence, decreased sanitizer efficacy during the flume tank washing of diced onions may be partly due to the protection of internalized *Listeria* cells during flume washing.

All studies have their own limitations based on the experimental design employed. In this work, the peeled onions that were first diced were also dip-inoculated to contain levels of *L. monocytogenes* unlikely to be found in naturally contaminated onions. However, high inoculation levels yielding quantifiable numbers of *Listeria* by direct plating were necessary for predictive modeling as demonstrated in previous studies. Commercial-type production practices for diced onions were followed as closely as possible using a commercial dicer and pilot-scale flume washing and dewatering line. However, differences in equipment design will affect the extent of bacterial cross-contamination [25]. During extended operation, any build-up of onion particulates would be expected to further extend the transfer of *Listeria.* In this study, the drum, blade, and chute of the dicer best supported the ongoing spread of *Listeria,* with these areas in need of more rigorous post-processing cleaning and sanitizing. Designers of such equipment should also pay special attention to cleanability and eliminate any potential harborage sites that may lead to biofilm formation and the long-term spread of microbial contaminants. Increasing levels of organic material in the flume water also dictate the need for continuous monitoring and control of the sanitizer concentration to maintain efficacy [26,36].

To our knowledge, this is the first study to show that *Listeria* can transfer and survive during the simulated commercial production of diced onions. During washing, the chlorine-based sanitizer used in this study was more effective at inactivating *Listeria* than peroxyacetic acid or electrolyzed water. However, organic matter commonly present in commercial onion wash water would be expected to decrease the efficacy of chlorine. Therefore, it is imperative that onions be diced and washed under as sanitary conditions as possible to minimize contamination, and then properly refrigerated to maximize end-product safety.

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References

1. United States Food and Drug Administration. Gills Onions Recalls One Lot of Diced Yellow Onions. 2007. Available online: https://wayback.archive-it.org/7993/20170112162847/http://www.fda.gov/Safety/Recalls/ArchiveRecalls/2007/ucm112229.htm (accessed on 14 September 2014).

2. United States Food and Drug Administration. Gills Onions Expands Voluntary Recall of Diced and Slivered Red and Yellow Onions, and Diced Onion and Celery Mix because of Possible Health Risk. 2007. Available online: https://wayback.archive-it.org/7993/20170112145217/http://www.fda.gov/Safety/Recalls/ArchiveRecalls/2012/ucm313399.htm (accessed on 14 September 2014).
3. California Department of Public Health. Gills Onions Listeria Mono. Available online: https://www.cdph.ca.gov/Programs/CEH/DFDCS/CDPH%20Document%20Library/FDB/FoodSafetyProgram/EnvInvReports/fdbEIRGO2012.pdf (accessed on 18 September 2014).
4. Centers for Disease Control and Prevention. Outbreak Investigation of *Salmonella*: Onions (October 2023). Available online: https://www.fda.gov/food/outbreaks-foodborne-illness/outbreak-investigation-salmonella-onions-october-2023 (accessed on 23 January 2024).
5. Beuchat, L.R. Pathogenic microorganisms associated with fresh produce. *J. Food Prot.* **1996**, *59*, 204–216. [CrossRef]
6. Food Safety News. *Listeria* Prompts Recall of Fresh-Cut Vegetables in 9 States. 2016. Available online: http://www.foodsafetynews.com/2016/08/listeria-prompts-recall-of-fresh-cut-vegetables-in-9-states/#.WUK0AWwkueB (accessed on 15 June 2017).
7. Sizmur, K.; Walker, C.W. *Listeria* in prepacked salads. *Lancet* **1988**, *331*, 1167. [CrossRef]
8. Denis, N.; Zhang, H.; Leroux, A.; Trudel, R.; Bietlot, H. Prevalence and trends of bacterial contamination in fresh fruits and vegetables sold at retail in Canada. *Food Control* **2016**, *67*, 225–234. [CrossRef]
9. Kaneko, K.; Hayashidani, H.; Takahashi, K.; Shiraki, Y.; Limawongpranee, S.; Ogawa, M. Bacterial Contamination in the environment of food factories processing ready-to-eat fresh vegetables. *J. Food Prot.* **1999**, *7*, 800–804. [CrossRef]
10. United States Food and Drug Administration. A&M Farms Initiates a Recall of Specific Little Bear Brand Whole Vidalia Onions Sold by the Pound at Retail Because of Possible Health Risk. Available online: https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/am-farms-initiates-recall-specific-little-bear-brand-whole-vidalia-onions-sold-pound-retail-because (accessed on 23 January 2024).
11. Buchholz, A.L.; Davidson, G.R.; Marks, B.P.; Todd, E.C.D.; Ryser, E.T. Quantitative transfer of *Escherichia coli* O157:H7 to equipment during small-scale production of fresh-cut leafy greens. *J. Food Prot.* **2012**, *75*, 1184–1197. [CrossRef]
12. Dunn, L.; Friedrich, L.M.; Strawn, L.K.; Danyluk, M.D. Prevalence of *Listeria monocytogenes* and indicator microorganisms in Florida cantaloupe packinghouses, 2013–2014. *Food Microbiol.* **2022**, *104*, 103970. [CrossRef]
13. Estrada, E.M.; Hamilton, A.; Sullivan, B.B.; Wiedmann, M.; Critzer, F.J.; Strawn, L.K. Prevalence, persistence, and diversity of *Listeria monocytogenes* species in produce packinghouses in three U.S. states. *J. Food Prot.* **2020**, *83*, 277–286. [CrossRef]
14. Ruiz-Llacsahuanga, B.; Hamilton, A.; Zaches, R.; Hanrahan, I.; Critzer, F. Prevalence of *Listeria* species on food contact surfaces in Washington state apple packinghouses. *Appl. Envoron. Microbiol.* **2021**, *87*, e02932-20. [CrossRef] [PubMed]
15. Sullivan, G.; Wiedmann, M. Detection and prevalence of *Listeria* in U.S. packinghouses and fresh-cut facilities. *J. Food Prot.* **2020**, *83*, 1656–1666. [CrossRef] [PubMed]
16. Townsend, A.; Strawn, L.K.; Chapman, B.J.; Dunn, L.L. A systematic review of *Listeria* species and *Listeria monocytogenes* prevalence, persistence, and diversity throughout the fresh produce supply chain. *Foods* **2021**, *10*, 1427. [CrossRef] [PubMed]
17. Xie, Y.; Nitin, N.; Harris, L.J. Transfer of *Enterococcus faecium* and *Salmonella enterica* during simulated postharvest handling of yellow onions (*Allium cepa* L.). *Food Microbiol.* **2023**, *115*, 104340. [CrossRef] [PubMed]
18. Oladimeji, V.; Jeong, S.; Almenar, E.; Marks, B.P.; Vorst, K.L.; Brown, B.; Ryser, E.T. Predicting the growth of *Listeria monocytogenes* and *Salmonella* Typhimurium growth in diced celery, onions, and tomatoes during simulated commercial transport, retail storage, and display. *J. Food Prot.* **2019**, *82*, 287–300.
19. Page, N.; González-Buesa, J.; Ryser, E.T.; Harte, J.; Almenar, E. Interactions between sanitizers and packaging gas compositions and their effects on the safety and quality of fresh-cut onions (*Allium cepa* L.). *Int. J. Food Microbiol.* **2016**, *208*, 105–113. [CrossRef] [PubMed]
20. Buchholz, A.L.; Davidson, G.R.; Marks, B.P.; Todd, E.C.D.; Ryser, E.T. Transfer of *Escherichia coli* O157:H7 from equipment surfaces to fresh-cut leafy greens during processing in a model pilot-plant production line with sanitizer-free water. *J. Food Prot.* **2012**, *75*, 1920–1929. [CrossRef]
21. Nou, X.; Luo, L. Whole leaf wash improves chlorine efficacy for microbial reductions and prevents cross-contamination during fresh-cut lettuce processing. *J. Food Sci.* **2010**, *75*, M283–M290. [CrossRef] [PubMed]
22. Kaminski, C.N.; Davidson, G.R.; Ryser, E.T. *Listeria monocytogenes* transfer during mechanical dicing of celery and growth during subsequent storage. *J. Food Prot.* **2014**, *77*, 765–771. [CrossRef]
23. Chen, D.; Zhao, T.; Doyle, M.P. Transfer of foodborne pathogens during mechanical slicing and their inactivation by levulinic acid-based sanitizer on slicers. *Food Microbiol.* **2014**, *38*, 263–269. [CrossRef]
24. Scollon, A.M.; Wang, H.; Ryser, E.T. Transfer of *Listeria monocytogenes* during mechanical slicing of onions. *Food Control* **2016**, *65*, 160–167. [CrossRef]
25. Wang, H.; Ryser, E.T. Quantitative transfer of *Salmonella* during mechanical slicing of tomatoes as impacted by multiple processing variables. *Int. J. Food Microbiol.* **2016**, *234*, 76–82. [CrossRef]
26. Wang, H.; Ryser, E.T. Quantitative transfer and sanitizer inactivation of *Salmonella* during simulated commercial dicing and conveying of tomatoes. *Food Control* **2020**, *107*, 106762. [CrossRef]

27. Aguado, V.; Vitas, A.I.; García-Jalón, I. Characterization of *Listeria monocytogenes* and *Listeria innocua* from a vegetable processing plant by RAPD and REA. *Intern. J. Food Microbiol.* **2004**, *90*, 341–347. [CrossRef]
28. Pérez-Rodríguez, F.; Valero, A.; Todd, E.C.D.; Carrasco, E.; García-Gimeno, R.M.; Zurera, G. Modeling transfer of *Escherichia coli* O157:H7 and *Staphylococcus aureus* during slicing of a cooked meat product. *Mear Sci.* **2007**, *76*, 692–699. [CrossRef]
29. Sheen, S.; Hwang, C.A. Mathematical modeling the cross-contamination of *Escherichia coli* O157:H7 on the surface of ready-to-eat meat product while slicing. *Food Microbiol.* **2010**, *27*, 37–43. [CrossRef]
30. Sheen, S.; Hwang, C.A. Modeling the surface cross-contamination of *Salmonella* spp. on ready-to-eat meat via slicing operation. *Food Nutr. Sci.* **2011**, *2*, 916–924.
31. Vorst, K.L.; Todd, E.C.D.; Ryser, E.T. Improved quantitative recovery of *Listeria monocytogenes* from stainless steel surfaces using a one-ply composite tissue. *J. Food Prot.* **2004**, *67*, 2212–2217. [CrossRef]
32. Buchholz, A.L.; Davidson, G.R.; Marks, B.P.; Todd, E.C.D.; Ryser, E.T. Tracking an *Escherichia coli* contaminated batch of leafy greens through a pilot-scale processing line. *J. Food Prot.* **2014**, *77*, 1487–1494. [CrossRef] [PubMed]
33. Alnughaymishi, H. *Listeria monocytogenes* Transfer during Slicing as Impacted by Intrinsic Characteristics of Fresh Produce. Ph.D. Thesis, Michigan State University, East Lansing, MI, USA, 2018.
34. Lundén, J.M.; Autio, T.J.; Korkeala, H.J. Transfer of persistent *Listeria monocytogenes* contamination between food-processing plants associated with a dicing machine. *J. Food Prot.* **2002**, *65*, 1129–1133. [CrossRef] [PubMed]
35. Gil, M.I.; Selma, M.V.; López-Gálvez, F.; Allende, A. Fresh-cut product sanitation and wash water disinfection: Problems and solutions. *Intern. J. Food Microbiol.* **2009**, *134*, 37–45. [CrossRef]
36. Davidson, G.R.; Buchholz, A.L.; Ryser, E.T. Efficacy of commercial produce sanitizers against nontoxigenic *Escherichia coli* O157:H7 during processing of iceberg lettuce in a pilot-scale leafy green processing line. *J. Food Prot.* **2013**, *76*, 1838–1845. [CrossRef] [PubMed]
37. Fatemi, P.; Frank, J.F. Inactivation of *Listeria monocytogenes*/*Pseudomonas* biofilms by peracid sanitizers. *J. Food Prot.* **1999**, *62*, 761–765. [CrossRef] [PubMed]
38. Gonzalez, R.J.; Luo, Y.; Ruiz-Cruz, S.; McEvoy, J.L. Efficacy of sanitizers to inactivate *Escherichia coli* O157:H7 on fresh-cut carrot shreds under simulated process water conditions. *J. Food Prot.* **2004**, *67*, 2375–2380. [CrossRef] [PubMed]
39. Tomás-Callejas, A.; López-Gálvez, F.; Sbodio, A.; Artés, F.; Artés-Hernández, F.; Suslow, T.V. Chlorine dioxide and chlorine effectiveness to prevent *Escherichia coli* O157:H7 and *Salmonella* cross-contamination on fresh-cut Red Chard. *Food Control* **2012**, *23*, 325–332. [CrossRef]

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