Food Microbiology 70 (2018) 120–128

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Association between bacterial survival and free chlorine concentration during commercial fresh-cut produce wash operation

Yaguang Luo [a, *], Bin Zhou [a], Sam Van Haute [b], Xiangwu Nou [a], Boce Zhang [a], Zi Teng [b], Ellen R. Turner [a, b], Qin Wang [b], Patricia D. Millner [a]

[a] U. S. Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, Environmental Microbiology and Food Safety Laboratory, 10300 Baltimore Ave, Beltsville, MD 20705, USA
[b] Department of Nutrition and Food Science, University of Maryland, 0112 Skinner Building, College Park, MD 20742, USA

ARTICLE INFO

Article history:
Received 27 February 2017
Received in revised form
14 September 2017
Accepted 21 September 2017
Available online 22 September 2017

Keywords:
Fresh produce
Wash water
Cross-contamination
APC
Chlorine
Disinfection
COD

ABSTRACT

Determining the minimal effective free chlorine (FC) concentration for preventing pathogen survival and cross-contamination during produce washing is critical for developing science- and risk-based food safety practices. The correlation between dynamic FC concentrations and bacterial survival was investigated during commercial washing of chopped Romaine lettuce, shredded Iceberg lettuce, and diced cabbage as pathogen inoculation study during commercial operation is not feasible. Wash water was sampled every 30 min and assayed for organic loading, FC, and total aerobic mesophilic bacteria after chlorine neutralization. Water turbidity, chemical oxygen demand, and total dissolved solids increased significantly over time, with more rapid increases in diced cabbage water. Combined chlorine increased consistently while FC fluctuated in response to rates of chlorine dosing, product loading, and water replenishment. Total bacterial survival showed a strong correlation with real-time FC concentration. Under approximately 10 mg/L, increasing FC significantly reduced the frequency and population of surviving bacteria detected. Increasing FC further resulted in the reduction of the aerobic plate count to below the detection limit (50 CFU/100 mL), except for a few sporadic positive samples with low cell counts. This study confirms that maintaining at least 10 mg/L FC in wash water strongly reduced the likelihood of bacterial survival and thus potential cross contamination of washed produce.

Published by Elsevier Ltd.

1. Introduction

Fresh fruits and vegetables are nutrient-rich foods with high levels of minerals, vitamins, and phytochemicals. However, recent outbreaks of food-borne illness associated with fresh produce have negatively impacted consumer confidence in the safety of fresh and fresh-cut produce (Lynch et al., 2009; Callejon et al., 2015; Arnade et al., 2015; Jung et al., 2014; Painter et al., 2013). Produce can be contaminated with pathogens during primary production and is often consumed raw without a 'kill step' such as cooking (Bartz et al., 2017). No effective or practical disinfection technologies are currently available to eliminate pathogens without significantly degrading produce quality, and post-harvest pathogen cross-contamination can significantly increase the size of foodborne illness outbreaks (Gil et al., 2009).

Washing is a critical process in preparing for fresh-cut "ready-to-eat" food product and is often the only step that could remove foreign materials and tissue exudates, and inactivate pathogens (Gil et al., 2009). Most fresh-cut produce washing is conducted by immersing produce in tanks or flumes of wash water, which is recirculated and reused due to the need for cost reduction and water conservation (Gil et al., 2009). During this process, pathogens dislodged from contaminated produce can survive in wash water, and spread to other clean produce that are washed simultaneously or subsequently in the same process water, causing pathogen cross-contamination of a large quantity of produce, if uncontrolled (Holvoet et al., 2012; López-Gálvez et al., 2009, 2010; Pérez-Rodríguez et al., 2014). Thus, the presence of sanitizers is critical to prevent pathogen survival and cross-contamination in fresh-cut produce washing operations (Luo et al., 2011; Tomas-Callejas et al., 2012).

Among available sanitizers, chlorine is most widely used in the fresh and fresh-cut produce industry due to its low cost, ease of use,

* Corresponding author.
E-mail address: Yaguang.Luo@ars.usda.gov (Y. Luo).

https://doi.org/10.1016/j.fm.2017.09.013
0740-0020/Published by Elsevier Ltd.

and its effectiveness against vegetative bacteria and some enteric viruses (Gombas et al., 2017; Van Haute et al., 2013; 2015; 2017). Traditionally, a free-chlorine concentration of 1 mg/L has been considered as the "Control Limit" and "rewash" as the "Corrective Action" in the hazard analysis and critical control points (HACCP) programs (Hurst, 2002; IFPA, 2001). Earlier research from Luo et al. (2011) demonstrated that this minimum FC concentration is inadequate to prevent pathogen survival and cross-contamination, and that rewashing is ineffective as a corrective action once produce has become contaminated. Luo et al. (2011) also reported that no cross-contamination was found when the FC concentration was at least 10 mg/L. Shen (2014) evaluated the effect of residual FC (i.e., measured free and available chlorine concentration after chlorine reaction with organic load) on pathogen cross-contamination and reported no pathogen cross-contamination during a decline in residual FC from 40.8 to 9.4 mg/L, but detectable cross-contamination after the FC declined to 4.6 mg/L and below. Gómez-López et al. (2014) used a different approach to evaluate pathogen survival during the dynamic changes in FC concentration affected by adding *Escherichia coli* O157:H7 inoculated spinach juice and continuously replenishing chlorine. When a free available chlorine concentration of 5 mg/L was maintained, no pathogens were detected in the wash water during the entire 1-h testing period. The reasons for these reported different results are unclear. While testing conditions in these reports were certainly different, future research will also need to determine if chlorine depletion cycles vs periodic replenishment vs. continuous replenishment have any impact on pathogen survival at the same level of free chlorine concentration.

Maintaining a stable FC concentration during fresh-cut produce washing is challenging, although residual FC is relatively stable in pure water (Suslow, 1997; Luo, 2007; Luo et al., 2011, 2012). Cut produce release copious amount of organic materials that quickly react with and deplete FC (Luo, 2007; Luo et al., 2012). Although the FC concentration can be restored to some extent through frequent addition of sodium hypochlorite, increased rate of addition is required as the accumulation of organic materials progresses over time. When the wash water's organic load gets too high, not only does the repeated chlorine addition become ineffective to restore FC concentration, the formation of hazardous chlorine byproducts, including trihalomethanes also causes concerns (Connell, 1996). The formation of hazardous chlorine off-gas if overdosing occurs together with excessively low pH may necessitate the temporary closure of a processing plant and the evacuation of employees (Connell, 1996). Furthermore, system water volume to product ratio and how/where chlorine is added to the water all impact the propensity to "gas-off". Thus, it is critical to determine the minimal FC residual that effectively prevents pathogen cross-contamination and also is feasible for commercial implementation.

In recognizing the food safety needs and practical challenges of maintaining sufficient sanitizer concentration during commercial fresh-cut produce wash operations, a working group consisting of technical experts from the produce industry, US government researchers, and scientists from academic fields has recently developed a guidance document entitled "Guidelines to validate control of cross-contamination during washing of fresh-cut leafy vegetables" (Gombas et al., 2017). One critical research need identified by the authors is specific scientific data obtained under commercial operating conditions to determine the minimal FC concentration required to prevent pathogen cross-contamination and the assessment of industry's process capability to maintain such FC concentration. Given that inoculating produce with human pathogens or even their toxin-free surrogates during commercial operations is not feasible due to the inherent food safety risks, assessment of the inactivation/survival of indigenous bacteria in wash water as impacted by real time FC concentration in a commercial setting may provide valuable insight.

In this paper, we report the first study to investigate the dynamic changes in organic load, pH and FC concentration, and the relationship between bacterial survival and the real time FC concentration, during commercial fresh-cut produce wash operations while processing major fresh-cut commodities including chopped Romaine lettuce, shredded Iceberg lettuce, and diced cabbage.

2. Materials and methods

2.1. Fresh-cut produce washing system

The experiment was conducted in collaboration with a medium-sized US fresh-cut produce processing facility during their routine commercial production. All tests were coordinated between the processor and researchers to ensure that all testing conditions were as consistent as possible with minimal disruption to the regular production flow. The wash system consists of a sequential double flume configuration, with wash water from individual flumes recirculating through a catch tank with a 1 mm screen for large debris removal and free chlorine replenishment (Fig. 1). The primary flume (flume A) has a capacity of 9000 L and the secondary (flume B) a capacity of 7100 L. The flume is filled with pre-chilled water (4 °C) before production starts with additional chilled water added to both flumes to compensate for processing water loss. A portion of the wash water in flume A is replaced periodically to avoid the excessive accumulation of organic load during operation. The FC and pH are adjusted with sodium hypochlorite and a phosphoric acid-based acidulant, respectively to approximate targeted values (Luo et al., 2012; Nou and Luo, 2010; Shen et al., 2012). The pH and FC level in the flumes were monitored and maintained using an Automated Analytic Platform™ (SmartWashSolutions Inc, Salinas, CA, USA).

2.2. Materials and processing

Romaine lettuce (*Lactuca sativa* var. *longifolia*), Iceberg lettuce (*Lactuca sativa* var. *capitata*), and cabbage (*Brassica oleracea* var. *capitata*) were harvested, stored at 5 °C and used within two days of harvesting. Fresh Romaine lettuce was trimmed onsite, and cut into 25 × 25 mm pieces (chop) using a belt-fed slicer (TranSlicer® 2510 Cutter, Urschel Laboratories, Inc., Chesterton, IN, USA) and introduced at an average rate of 1560 kg/hr into flume A. Iceberg lettuce was pre-cored in the field, cut into 6 mm strips (shred) using TranSlicer® 2510 Cutter (Urschel Laboratories) and introduced at an average rate of 3660 kg/hr into flume A. Cabbage was trimmed and cored onsite and cut into 5 × 5 mm pieces (dice) using a DiversaCut (Urschel Laboratories) and introduced at an average rate of 2240 kg/hr into flume A. Cut vegetables were sequentially washed in flumes A and B, each with a residence time of about 30 s. Three independent runs of the washing operation were conducted for each type of produce.

2.3. Sample collection

For each independent run, Wash water samples were collected at two locations in each flume (Fig. 1) in 30-min intervals for up to approximately 2.5 h production schedule of the selected products. FC and total chlorine were determined immediately upon sample collection, and the other water quality parameters were tested in an auxiliary laboratory at the facility. Parallel samples were taken using sterile containers for microbiological analyses. For Romaine lettuce, the microbial data from the first trial was not included due to issues encountered in setting up the mobile microbial lab. So, only 2 repetitions of the microbial data were used for chopped