Food Control 25 (2012) 745–751

Contents lists available at SciVerse ScienceDirect

Food Control

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

The effect of different processing parameters on the efficacy of commercial post-harvest washing of minimally processed spinach and shredded lettuce

Maria J. Barrera [a], R. Blenkinsop [b], K. Warriner [a,*]

[a] Department of Food Science, University of Guelph, Guelph, ON N1G 2W1, Canada
[b] Ontario Ministry of Agriculture, Food and Rural Affairs, 1 Stone Road W., 5th Floor NW, Guelph, ON N1G 4Y2, Canada

ARTICLE INFO

Article history:
Received 19 July 2011
Received in revised form
6 December 2011
Accepted 10 December 2011

Keywords:
Fresh produce
Spinach
Lettuce
Wash water
Oxidation–reduction potential
Peroxyacetic acid
Escherichia coli
Coliforms
Decontamination
Post-harvest

ABSTRACT

The effect of different processing parameters on the efficacy of commercial post-harvest biocidal washes to decrease the bacterial loading on spinach and lettuce has been evaluated. Sampling was performed at two spinach processors (Facility A & B) and a shredded lettuce producer (Facility C). Aerobic colony counts (ACC) and coliform counts were determined on samples taken at pre- and post-wash. In parallel, the heterotrophic plate count (HPC) and coliform levels in wash water was also determined. Processing parameters measured were the temperature of leafy greens (pre- and post-washing) and wash water. The sanitizer levels (peroxyacetic acid, oxidation–reduction potential), pH, conductivity and turbidity were also measured. The wash process in Facility B had a residence time of 50 s for the spinach, maintained a constant hypochlorite concentration and continuously re-charged the tanks with fresh water. In contrast, Facility A had a short residence time (15 s) did not maintain a constant sanitizer (peroxyacetic acid) concentration or re-charge tanks with fresh water. Despite the differences in processing operations there was no statistical difference between the log count reductions (LCR) obtained in ACC and coliform counts although counts were only reduced by <0.6 log cfu/g. The carriage of *Escherichia coli* on pre-wash spinach was 19% and 25% in Facility A and B respectively. There was a high prevalence (57% positive) of *E. coli* in the wash water of Facility A with none being recovered in water samples taken from Facility B. Yet, the carriage of *E. coli* on post-wash spinach was the same in the two facilities (7%). Lettuce harboured a lower level of both ACC and coliforms with LCR being significantly greater than spinach. In general, the LCR in ACC and coliforms could be positively correlated to bacterial counts of pre-washed leafy greens and conductivity (solids content) of the wash water. A negative correlation was found between LCR and water temperature. Interestingly, within the ranges measured the LCR was independent of the bacterial loading of the water. The results of the study confirmed the limited efficacy of biocidal washes to remove field acquired contamination. Although it is thought maintaining a low microbial loading in the wash water and maintaining sanitizer concentration is key the current study suggests high conductivity and low temperature of the wash water enhances the LCR achieved.

1. Introduction

Although fresh-cut produce such as bagged leafy green salads offer convenience and extended shelf-life there has be numerous foodborne illness outbreaks associated with such products (Berger et al., 2010; Critzer & Doyle, 2010; Newell et al., 2010; Warriner, Huber, Namvar, Fan, & Dunfield, 2009). A diverse range of human pathogens have been associated with outbreaks linked to leafy vegetables although *Salmonella* and *Escherichia coli* O157:H7 are commonly implicated (Critzer & Doyle, 2010). The most notable outbreak occurred in 2006 that implicated bagged spinach contaminated with *E. coli* O157:H7. Here, it was thought that the spinach became contaminated in the field and carried through to the final consumer. The outbreak resulted in 205 confirmed cases and 3 deaths although the actual number of those affected is likely to be higher given that the outbreak was spread over 22 US States with cases also being reported in Canada (Wendel et al., 2009).

In the course of cultivation the developing plant is open to contamination from a wide variety of sources that includes manure amended soil, irrigation water, insects and wild animals (Warriner et al., 2009). A wash step is applied in fresh-cut processing in an attempt to remove field acquired contamination or at least prevent cross-contamination between batches (Gil, Selma, Lopez-Galvez, &

* Corresponding author. Tel.: +1 519 8244120; fax: +1 519 8246631.
E-mail address: kwarrine@uoguelph.ca (K. Warriner).

0956-7135/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodcont.2011.12.013

Allende, 2009; Nou et al., 2011). A diverse range of sanitizers have been applied in produce washing with 100 ppm hypochlorite remaining the most commonly used (Erkmen, 2010). Because hypochlorite is rapidly sequestered by organic material it is common practice in industry to apply an oxidation–reduction potential (ORP) control system (Gonzalez, Luo, Ruiz-Cruz, & McEvoy, 2004). Here, the ORP is maintained at a set point by the addition of hypochlorite and citric acid. Therefore, in theory at least, the active hypochlorous acid concentration is held constant regardless of the organic loading in the wash water. Peroxyacetic acid is an alternative sanitizer with the advantage over hypochlorite in being relatively stable in the presence of organic material (Beuchat, Adler, & Lang, 2004; Hellstrom, Kervinen, Lyly, Ahvenainen-Rantala, & Korkeala, 2006; Lopez-Galvez, Allende, Selma, & Gil, 2009; Vandekinderen, Devlieghere, De Meulenaer, Ragaert, & Van Camp, 2009; Zhang, Ma, Phelan, & Doyle, 2009). Peroxyacetic acid is recommended to be used at levels in the order of 50 ppm although typically lower concentrations (5–30 ppm) are applied due to cost (Vandekinderen et al., 2009).

Although there have been numerous reports on the efficacy of sanitizers to decontaminate fresh produce these have been restricted to laboratory trials were conditions are controlled. Commercial wash processes are more dynamic with respect to the microbial loading on the incoming raw material and water parameters (temperature, sanitizer concentration, organic (conductivity) and microbial loading (Vandekinderen et al., 2009)). There remains a key knowledge gap with respect to which parameters most significantly impact on the efficacy of the wash process. It can be envisaged that if wash parameter(s) that affect the LCR were known then these could be used to optimize wash conditions. Therefore, the objective of the following study was to identify factors that affect the log count reduction (LCR) achieved in three commercial leafy green processing operations.

2. Materials and methods

2.1. Facility description

Three different facilities were visited in the course of the study. Facility A, processed 451 kg spinach per hour over a processing period of 5–8 h. The spinach was manually loaded from containers onto an elevator conveyor which transported the leaves to a rotating de-stoner unit. The spinach then passed through an inspection belt prior to entering the first of two wash tanks. The first wash tank contained 1000 l of municipal water and functioned to remove the gross organics (for example, soil) on the spinach prior to being transferred to a second water tank (same volume) manually dosed with peroxyacetic acid (Tsunami 100®; Ecolab, Minnesota, USA) every 3 h. The residence time of the spinach within each wash tank was approximately 15 s with no re-charging of the water during the processing activity. The washed spinach was de-watered via centrifugation prior to being placed into packs and sealed.

Plant B processed 1300 kg spinach per hour with a processing activity lasting between 4 and 8 h. The spinach was loaded from a hopper onto a conveyor which transported the product to two wash tanks each with a capacity of approximately 4800 l. The resident time within each wash tank was 50 s with the hypochlorite (hypochlorous acid concentration) being maintained via an ORP feedback loop with a set point of 850 mV and pH 6.8 (adjusted with citric acid). The water in the wash tank was continuously replenished throughout the processing activity. Spinach was then transported to an infrared drying cabinet prior to packing.

Plant C processed 2300 kg shredded iceberg lettuce per hour over a processing period of up to 30 h. The lettuce heads were loaded onto a conveyor and passed through a shredder that empted the product into a closed water flume that transported the lettuce to a 6000 l volume wash tank that was continuously replenished with fresh water. The lettuce was washed for 3 min in hypochlorite solution poised at 650–800 mV and pH 6.8–7.5. The washed lettuce was de-watered prior to being packed.

2.2. Sample collection

Samples were taken at different time periods throughout the processing activity to give variation in the measured parameters. Each sample set consisted of 300 g leafy greens taken immediately before entering the wash tank or flume (pre-wash) and a further batch before entering to de-watering unit (post-wash). Water samples (2 × 250 ml) were taken from the biocidal (second) wash tank. The temperature of the produce (pre- and post-wash) and wash water was taken using a digital thermometer (Thermo Fisher, Oakville, ON, Canada). The conductivity, pH and ORP of the wash water samples were measured using XL20 meter (Thermo Fisher) fitted with the appropriate probe. Peroxyacetic acid concentration of Facility A water samples was determined by using Peracid/Peroxide Test (Kit #311, EcoLab). Turbidity was determined for the three facilities by reading Absorbance of wash water at 540 nm using a SmartSpec™Plus Spectrophotometer (Bio-Rad Laboratories, Hercules, CA).

2.3. Microbiological analyses

Leafy green samples (25 g) were placed into 225 ml of 0.1% peptone water and stomached for 150 s. A dilution series was prepared and plated onto Aerobic Plate Count (Health Canada MFHPB-33) and *E. coli*/coliform (Health Canada MFHPB-43) Petrifilms (3 M Canada Inc., London, ON). Aerobic Count Plates were incubated at 35 °C for 48 h. The minimum detection limit (MDL) for both pre- and post-washed samples is 0.65 log cfu/g. *E. coli*/coliform Petrifilms were incubated at 35 °C for 24 h. MDL for pre- and post-washed products was 0.4 log cfu/g.

Heterotrophic plate count was enumerated on plate count agar incubated 35 °C for 48 h. Results were expressed as log cfu/ml. Total coliforms and *E. coli*, were enumerated in 100 ml volumes of wash water that was filtered through 47 mm diameter, 0.45 μm pore size membrane filters. The filter was placed on differential coliform (DC) agar plate and incubated at 37 °C for 24 h. Red and/or pink colonies are counted as coliforms, and blue/purple as *E. coli*.

2.4. Experimental design and statistics

In total 416 leafy green and 256 water samples were screened over the course of the study. Facility A was visited six times with a total of 192 spinach and 96 water samples being collected. Facility B was visited four times with 128 spinach and 64 water samples being screened. Finally, Facility C was visited on three occasions with 96 lettuce and 48 water samples being collected. Each sample analysis was performed in duplicate with the bacterial counts being transformed into $\log_{10}$ values prior to statistical analysis using ANOVA and Tukey Test. Correlations between log count reductions (LCR) of aerobic counts and coliforms were calculated using the Spearman's Correlation Coefficient. Relationship between LCR to the rest of the parameters required all data collected within the same visit and time to be treated as a continuous variable, and the covariance structure was taken into account using SAS® Proc Mixed for Windows, v.9.1, SAS Institute, Inc. (Cary, NC, USA).