Food Microbiology 73 (2018) 129–136

Contents lists available at ScienceDirect

Food Microbiology

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

Decontamination and survival of *Enterobacteriaceae* on shredded iceberg lettuce during storage

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ARTICLE INFO

Article history:
Available online 3 February 2018

Keywords:
Enterobacteriaceae
Decontamination
Sodium hypochlorite
Peroxyacetic acid
Ionizing radiation

ABSTRACT

Enterobacteriaceae family can contaminate fresh produce at any stage of production either at pre-harvest or post-harvest stages. The objectives of the current study were to i) identify *Enterobacteriaceae* species on iceberg lettuce, ii) compare the decontamination efficiency of water, sodium hypochlorite (free chlorine 200 ppm), peroxyacetic acid (PA 80 ppm; Kenocid 2100®) or their combinations and ionizing radiation against *Enterobacteriaceae* on shredded iceberg lettuce and iii) determine the survival of *Enterobacteriaceae* post-treatment storage of shredded iceberg lettuce at 4, 10 and 25 °C, for up to 7 days. *Klebsiella pneumonia* spp. *pneumonia*, *Enterobacter cloacae*, *Klebsiella oxytoca*, *Pantoea* spp., *Leclercia adecarboxylata* and *Kluyvera ascorbate* were identified on iceberg lettuce. No significant difference ($P \geq 0.05$) among *Enterobacteriaceae* survival after washing with water or sanitizing with sodium hypochlorite or Kenocid 2100® (reduction ≤ 0.6 log CFU/g) were found. Combined sanitizer treatments were more effective against *Enterobacteriaceae* than single washing/sanitizing treatments. Sanitization of iceberg lettuce with combined washing/sanitizing treatments reduced *Enterobacteriaceae* by 0.85–2.24 CFU/g. Post-treatment growth of *Enterobacteriaceae* during storage on samples sanitized with sodium hypochlorite and Kenocid 2100® was more than on samples washed with water. The D_{10}-value of *Enterobacteriaceae* on shredded iceberg lettuce was 0.21 KGy. The reduction of *Enterobacteriaceae* populations on iceberg after gamma radiation (0.6 KGy) was 3 log CFU/g, however, *Enterobacteriaceae* counts increased post-irradiation storage by 4–5 log CFU/g. Therefore, washing shredded iceberg lettuce with combined sanitizing treatment (sodium hypochlorite/sodium hypochlorite, sodium hypochlorite/Kenocid 2100®, or Kenocid 2100®/Kenocid 2100®) for total time of 6 min or exposing it to gamma irradiation (0.6 KGy) can decrease the risk of *Enterobacteriaceae* (reduction ≥ 2 log). Post-washing storage of sliced iceberg lettuce (4, 10, 25 °C) could increase the risk of *Enterobacteriaceae* as their counts increased during storage even at low temperatures.

1. Introduction

Consumption of fresh leafy green vegetables that are eaten raw or with minimal processing has increased because of healthy lifestyle patterns. Contamination of these products with microorganisms may be a health hazard to consumers and cause foodborne illness outbreaks. Leafy green vegetables may be contaminated with pathogenic and non-pathogenic bacteria at several points throughout the pre-harvest and post-harvest systems from irrigation water, feces, dust, soil, human handlings, storage facilities, distribution systems, processing equipment and poor hygienic conditions (Beuchat, 1996). These bacteria can attach to the surface of leafy green vegetables and survive for extended periods time (Brandl and Amundson, 2008; Delaquis et al., 2007). Iceberg lettuce is one of the produce commodities most susceptible to contamination. Doyle and Erickson (2008) noted that part of the plant closest to the soil contained higher concentration of the microbes.

Enterobacteriaceae is classified as large family of bacteria with common characteristics comprise of Gram-negative, rod-shaped, facultative anaerobic, non-spore forming, either capsulated or non-capsulated in addition of being motile or non-motile. Presence of pathogenic and antimicrobial resistant members of *Enterobacteriaceae* on fresh produce at retail constitute severe threats to consumers. Pathogenic or antimicrobial resistant members of

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https://doi.org/10.1016/j.fm.2018.01.022
0740-0020/

Enterobacteriaceae have been isolated from green leafy vegetables (Al-Kharousi et al., 2016; Al-Holy et al., 2013; Hassan et al., 2011). Al-Holy et al. (2013) and Hassan et al. (2011) isolated *Klebsiella pneumoniae*, *Enterobacter sp.*, *Escherichia coli*, *Citrobacter sp.*, *Acinetobacter*, *Shigella flexneri* and other *Enterobacteriaceae* members that are resistant to antibiotics from green leafy vegetables sold in Saudi Arabia.

Many methods have been used to decontaminate bacteria on fresh produce. Sodium hypochlorite (form of chlorine) has been widely used to reduce microbial contamination on fresh produce including lettuce. Escudero et al. (1999) reported that exposure of shredded lettuce to 100 and 300 ppm chlorine for 10 min reduced the populations of *Yersinia enterocolitica* 2 to 3 log CFU/g. However, other studies have found that chlorine (<200 ppm) are not effective at reducing microbial populations on lettuce (Li et al., 2001a; Taormina and Beuchat, 1999).

Peroxyacetic acid (PA) has also been widely used to reduce microorganisms on fresh produce. Masson (1990) found that 90 ppm PA reduced total counts and fecal coliforms on cut salad mixtures by 2 log CFU/g. However, Davidson et al. (2013) showed that 50 ppm PA (Tsunami® 100), and 50 ppm of mixed peracid (Tsunami® 200) were not significantly more effective than water at reducing of *E. coli* O157:H7 on iceberg lettuce.

Ionizing radiation maybe considered one of the most promising technology to improve the safety of fresh produce. In 2008, U.S. Food and Drug Administration (FDA) (2008) conferred its acceptance to use irradiation for killing pathogens on iceberg lettuce. Niemira (2007) compared between the effect of sodium hypochlorite wash and irradiation against *E. coli* O157:H7 internalized in romaine lettuce leaves. The author reported that the D_{10}-value of *E. coli* O157:H7 in lettuce leaves was 0.39 KGy, while sodium hypochlorite (chlorine 300 and 600 ppm) resulted in <1 log reduction and water wash was ineffective.

No studies were found in the literature on the effect of washing/sanitizing solutions (water, sodium hypochlorite, Kenocid 2100® (PA) or their combination) and ionizing radiation against *Enterobacteriaceae* on shredded iceberg lettuce during post treatment storage. Thus, the objectives of the current study were to i) identify *Enterobacteriaceae* species found on iceberg lettuce, ii) compare the decontamination efficiency of washing/sanitizing solutions (water, sodium hypochlorite, Kenocid 2100® (PA) or their combination) and ionizing radiation against *Enterobacteriaceae* on shredded iceberg lettuce and iii) determine post-treatment survival of *Enterobacteriaceae* on shredded iceberg lettuce during storage at 4, 10 and 25 °C for up to 7 days.

2. Materials and methods

2.1. Preparation of iceberg lettuce

Iceberg heads samples were purchased from a local market, at the day of each experiment. Damaged leaves were removed and only intact leaves were used in the experiments. Iceberg leaves samples of each experiment were prepared from five iceberg heads. The leaves were shredded manually (*ca* 1 × 3 cm) by sanitized knife and cutting board. The shredded leaves were mixed manually in a sterile bag to be used in the experiments (25 g quantities).

2.2. Determination of aerobic plate count and Enterobacteriaceae count on iceberg samples

Each sample (25g) was placed into sterile Stomacher bags (Seward, UK) under aseptic condition and combined with 225 ml quantities of peptone water (0.1%; Oxoid, UK), and then was homogenized in the Stomacher (AES-Chemunex, France) for 2 min. The samples were serially diluted in 9 ml of sterile peptone water (0.1%) and volumes of 0.1 ml of suitable dilutions were plated in duplicate on Nutrient agar (Oxoid) to enumerate total plate counts and in Violet Red Bile Glucose agar (VRBGA, Oxoid) by pour plate method to enumerate *Enterobacteriaceae*. The plates were incubated aerobically at 37 °C for 24 h. The typical *Enterobacteriaceae* colonies were counted expressed as log (CFU/g).

2.3. Identification of Enterobacteriaceae on the iceberg samples

To reveal *Enterobacteriaceae* members on iceberg lettuce, thirty morphological different *Enterobacteriaceae* colonies grown on/in Violet Red Bile Glucose agar (VRBGA) from different shredded iceberg samples were identified using the VITEK 2 automated system (bioMérieux S.A., Marcy L'Etoile, France). The ID-GNB card was used for *Enterobacteriaceae* identification.

2.4. Decontamination of iceberg lettuce

2.4.1. Washing/sanitation-single treatment

In this method, water washing (sterile), sodium hypochlorite and Kenocid 2100® were used separately to sanitize the shredded iceberg samples. Commercial sodium hypochlorite (NaOCl) was purchased from the local market. Free chlorine concentration was determined using the method described by Willson (1935). Sodium hypochlorite solution was freshly prepared prior to each experiment by diluting 5 ml NaOCl (free chlorine 4%) with 1000 ml of 0.05M potassium phosphate buffer (pH 6.8 at 25 °C) to achieve free chlorine concentration of 200 ppm (Lang et al., 2004). Kenocid 2100® (Ieper, Belgium), a peroxyacetic acid-based sanitizer, was obtained from the local market. It contains hydrogen peroxide (H_2O_2 20%), peracetic acid (PA 5%) and acetic acid (AA 10%). The solution was freshly prepared prior to each experiment to obtain PA concertation of 80 ppm. The solution was prepared by adding 1.6 ml of the Kenocid 2100® to 1000 ml sterile distilled water.

Shredded iceberg leaves (25g) were placed into sterile Stomacher bag and 100 ml of distilled water (at room temperature, 21±1 °C), sodium hypochlorite (chlorine 200 ppm) or Kenocid 2100® (PA 80 ppm) was added to the bag. After mixing thoroughly for 3 min, the solution was drained out from the bag and the leaves were dried by tissue paper to remove the remaining solution on the samples.

2.4.2. Washing/sanitation-combined treatment

In this part of the study, combinations of treatments were used [(washing with water followed by washing with water), (washing with water followed by sanitizing with sodium hypochlorite), (washing with water followed by sanitizing with Kenocid 2100®), (sanitizing with sodium hypochlorite followed by sanitizing with sodium hypochlorite), (sanitizing with sodium hypochlorite followed by sanitizing with Kenocid 2100®) and (sanitizing with Kenocid 2100® followed by sanitizing with Kenocid 2100®)]. Shredded lettuce sample (25g) was firstly treated with the first solution (distilled water, sodium hypochlorite or Kenocid 2100®) for 3 min. Then, the solution was drained out from the bag and the shredded lettuce was kept at room temperature for 5 min. Consequently, the second solution was added to the bag and mixed with the shredded lettuce thoroughly for 3 min. After that, the solution was drained out from the bag and the shredded lettuce samples were dried by tissue paper without water rinse to shorten the washing/sanitizing process.

2.4.3. Ionizing radiation

Quantities of 25 g of shredded lettuce was placed in sterile Stomacher bags, pressed by hand to remove air and sealed. The