International Journal of Food Microbiology 269 (2018) 27–35

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Outgraded produce variably retains surface inoculated *Escherichia coli* through washing

Shiyu Cai[a], Randy W. Worobo[a], Abigail B. Snyder[b,*]

[a] Department of Food Science, Cornell University, Ithaca, NY 14853, United States
[b] Department of Extension, The Ohio State University, Wooster, OH 44691, United States

ARTICLE INFO

Keywords:
Produce safety
Outgraded produce
Secondary quality
Food waste

ABSTRACT

The use of secondary quality produce has gained attention as a solution to food waste in both the U.S. and Europe. The purpose of this study was to evaluate the impact of using secondary quality or outgraded produce on the retention of surface inoculated *E. coli* following a rinse treatment on four model fresh produce systems (apple, tomato, carrot, lettuce). A three-strain cocktail of rifampicin-resistant generic *E. coli*, with a concentration of 9.0 log CFU/mL, was spot-inoculated on the intact surfaces of U.S. No.1 grade produce items and damaged or decayed areas of outgraded produce items. Generally, outgraded produce of all four kinds retained higher levels of inoculated *E. coli* following two postharvest treatments, chlorinated (150 ppm) or water only. However, physical damage, those defects which compromised the integrity of the produce surface, lead to significantly greater *E. coli* levels following rinsing than did physiological defects. Compared to U.S. No.1 quality apples, outgraded apples retained 4.3 ± 1.4 log CFU/g more *E. coli* following water only treatment, and 3.6 ± 1.7 log CFU/g more following chlorine treatment. Outgraded tomatoes retained significantly more (3.5 ± 1.1 log CFU/g) inoculated *E. coli* following water only rinse and 3.0 ± 1.4 log CFU/g more inoculated *E. coli* following chlorine treatment than U.S. No.1 quality tomatoes did under the same treatment conditions. Outgraded carrots retained 1 ± 1.1 log more CFU/g inoculated *E. coli* following water only treatment and 0.5 ± 0.8 log more CFU/g inoculated *E. coli* following chlorine treatment, compared to U.S. No.1 carrots. Outgraded lettuce leaves retained 1.6 ± 0.5 log CFU/g more inoculated *E. coli* following water only treatment and 4.1 ± 0.4 log CFU/g more inoculated *E. coli* following chlorine treatment than did U.S. No.1 quality lettuce leaves under the same treatment conditions. Treating with 150 ppm chlorine was not sufficient to eliminate the increased microbial retention associated with secondary quality or outgraded produce, and the efficacy of disinfection was greatly affected by type of defect. Apples with physical damage retained significantly higher *E. coli* loads than did those with physiological defects, an additional 2.6 log CFU/g under chlorine treatment and 0.8 log CFU/g more under was water only treatment. Tomatoes with physical damage had a 1.3-log CFU/g and 0.6-log CFU/g average increase of retained *E. coli* counts compared to those with physiological defects following a chlorine and water only treatment, respectively. Although a chlorine dip provided only a modest reduction in pathogens, generally, outgraded produce with physiological defects may present less food safety risks if introduced into the fresh market than does produce with physical damage due to their enhanced retention of bacterial cells. Therefore, as industry considers how to minimize its food waste problem, preferentially directing physically damaged produce away from the fresh market will help to minimize risk while maximizing food resources.

1. Introduction

According to the Food and Agriculture Organization of the United Nations (2011) on global food losses and food waste, roughly 45% of the fruits and vegetables (including roots and tubers) produced for human consumption are discarded, lost, or uneaten. Compared to all other food groups (meats, dairy, etc.) fruits and vegetables have the highest wastage rate. In the U.S., the USDA Agricultural Market Service grades produce based on certain quality characteristics and some markets require certain produce quality grades for sale or distribution. Although attributes vary by produce type, the U.S. No. 1 standard generally requires produce to be at least fairly smooth on the surface, fairly well colored, fairly well formed, and free from decay (see Table A.1 in Appendix for full descriptions). Fruits and vegetables that do not

* Corresponding author at: 1680 Madison Ave., Wooster, OH 44691, United States.
E-mail address: Snyder.814@osu.edu (A.B. Snyder).

https://doi.org/10.1016/j.ijfoodmicro.2018.01.012
Received 17 August 2017; Received in revised form 20 December 2017; Accepted 10 January 2018
Available online 12 January 2018
0168-1605/

meet the U.S. No. 1 standard are often outgraded from commercial sale. Out-grading of blemished, misshapen, or wrong-sized foods due to minimum quality standards set by the federal marketing orders (Powers, 1990) and consumer's expectation of cosmetic perfection lead to non-harvest and culling of edible produce. Even though outgraded fruits and vegetables are sometimes used for processing, most large processors in the United States themselves have product specifications (e.g. varieties specific for processing) which limit this waste recovery strategy (NRDC, 2012). In response to calls for food waste reduction, retailers are gradually bringing blemished fruits and vegetables into the fresh market. With the increasing sales and consumption of deformed and blemished fresh fruits and vegetables, there is a need to evaluate potential risks to food safety that may be introduced. This is especially relevant when considering that food insecure consumers are often more vulnerable to disease sequela associated with foodborne illness.

Fruits and vegetables consumed raw pose a food safety risk since no kill step is typically applied. The CDC reported that from 2002–2011, 667 outbreaks (17% of total) were associated with produce and 23,748 people (24% of total cases) in the United States were sickened from consuming contaminated fresh produce. The number of produce-associated outbreaks exceeded all other food types and caused, on average, the largest number of illnesses per outbreak (DeWaal and Glassman, 2014). Many produce commodities are susceptible to contamination from soil, irrigation water, wild and domestic animals, and inadequately composted manure prior to harvest (Cooley et al., 2007; Jay et al., 2007). U.S. produce packers and the fresh-cut industry commonly use a triple-wash technology with low concentrations of chlorine, peracetic acid, or other sanitizing agents to reduce the incidence of cross-contamination and improve the safety of their products (Parish et al., 2003). These treatments are known to remove around 1–2 log CFU/g of microbial pathogens and prevent cross-contamination through contaminated wash water (Akbas and Olmez, 2007; Lee et al., 2014; Snyder et al., 2016).

However, the efficacy of sanitizers and washing on bacteria removal depends on the type of produce and contamination sites. Cracks in the produce surface, stem stars, and damaged tissue may protect pathogens from removal, and retained pathogens may contaminate edible parts during cutting, slicing and peeling (Olaimat and Holley, 2012). Alvarado-Casillas et al. (2007), Wang et al. (2009), and Snyder et al. (2016) have reported that disinfection treatment inactivates various levels of pathogens on different types of produce due to variably unique surface roughness. Surface topographies vary among different types of produce, but also among different units of the same produce type. Surface topography can provide a protective environment for pathogens against decontamination treatments, and retain more pathogens than smooth intact surfaces (Seo and Frank, 1999; Han et al., 2001; Yuk et al., 2005; Fatemi and Knabel, 2006; Felkey et al., 2006; Aruscavage et al., 2008). In addition, internalization occurs during post-harvest cooling and washing steps where water may be a vehicle for pathogen internalization through deformed sites, and temperature differentials cause surface-borne microbes to ingress through wounds, lesions, and stomata. Burnett et al. (2000) has found that bacterial cells can penetrate at a depth up to 70 μm through damaged tissues surrounding puncture wounds. Once pathogen cells penetrate deeper than 30 to 40 μm from the damaged plant tissue surface, sanitizer treatments are virtually ineffective (Fatemi and Knabel, 2006). The degree to which these defects protect bacterial contaminants likely varies by the type and severity of damage. Relatedly, Wei et al. (1995) suggested the effectiveness of such protection depends on the cause of the damage (e.g. physical wounding, pest damage, or plant disease). Shallow splits to the fruit cuticle may not harbor microbes to the same extent as those that extend through to the interior tissue of the fruit. Additionally, the exposure of plant nutrients may facilitate outgrowth of pathogens, while the application of antimicrobials may have decreased efficacy.

In order to reduce fresh produce waste and increase access among food insecure populations, risk identification and proper risk management strategies are needed to protect food safety. This study evaluated the efficacy of wash treatments to decontaminate secondary quality or outgraded produce with surface inoculated *E. coli* on four model fresh produce systems.

2. Materials & methods

2.1. Bacterial strains & inoculum preparation

A cocktail of rifampicin resistant derivatives of generic *E. coli* strains was used to inoculate produce. The cocktail contained TVS 353 (derived from *E. coli* W778), TVS 354 (derived from *E. coli* P149) and TVS 355 (derived from *E. coli* S19) as described by Tomás-Callejas et al. (2011). These strains were originally isolated from surface irrigation water, Romaine lettuce, and sandy-loam soil samples (Salinas Region, CA, USA), respectively, and have been utilized as model pathogens in fresh produce production systems (Tomás-Callejas et al., 2011). Bacterial cultures were stored at −80 °C in Tryptic Soy Broth (TSB) (Becton, Dickinson and Co., Sparks, MD) containing 25% (vol/vol) glycerol. To prepare the inoculum, the three generic *E. coli* strains were grown separately in 9 mL TSB supplemented with 100 mg/L of rifampicin (EMD Millipore Corp., Billerica, MA), and incubated at 37 °C for 18 h on a rotary shaker (200 RPM). After incubation, *E. coli* cells were harvested by centrifugation (13,000 RPM, 10 min), re-suspended after being washed twice in phosphate-buffered saline (PBS, pH 7.0, Fisher Chemical, Inc., Fair Lawn, NJ). The three bacterial suspensions were combined and the final concentration of the inoculum was determined by plating on Tryptic Soy Agar supplemented with 100 mg/L of rifampicin (TSA-rif) to be about 9.0 log CFU/mL.

2.2. Fresh produce selection & grading

Four types of fresh produce, tomatoes (BHN 589, Cornell University, Geneva, NY), apples (Cortland, Gala, Honeycrisp, Cornell University, Ithaca, NY), carrots (Imperator, purchased from a specialty grower-seller, Ithaca, NY) and whole-head lettuce (Romaine, purchased from a commercial retailer, Ithaca, NY), were selected to represent a wide variety of common fruits and vegetables in the market. For tomatoes, apples, and carrots, 100 items containing 50 U.S. No.1 quality items and 50 secondary culls were obtained. For Romaine lettuce, ten whole heads of lettuce were obtained from the same lot at a commercial retailer (Ithaca, NY). Produce items were collected throughout October and November 2016. All the selected produce items were held at 4 °C for up to 48 h until use.

Tomatoes, apples, carrots, and lettuce were graded categorically as “U.S. No.1”, “Injury”, “Damage” and “Serious Damage” according to the USDA Market Inspection Instructions (USDA, 2004a, 2004b; USDA, 2005a, 2005b) by trained researchers, and the assignment of degree and type of defect was verified by an independent fruit/vegetable physiologist. For each experimental condition, 25 U.S. No.1 and 25 outgraded (Injury, Damage, Serious damage) produce items were selected, but the numbers of each grade were not controlled. Type of defects for tomatoes included growth cracks, catfaces, zippers, bruises, insect stings, and shapes (USDA, 2005b). Defects on apples were mainly russeting, insect stings, lesions, scald and shapes (USDA, 2005a). Russeting was caused by apple rust mites feeding on fruitlets, whereas insect stings were identified as damages caused after apple maturity. Forking or deformity with a few cracks/holes was observed in secondary quality carrots (Fig. 1). For lettuce, the outgraded quality leaves were taken from the exterior of the lettuce head and were characterized by blemishes and wilt, and are frequently removed by retailers before sale (Fig. 1). These defects were identified as physical damage and pink ribs (USDA, 2004b). Meanwhile, the internal lettuce leaves without any damage were considered the highest quality produce. Because selected produce was harvested throughout October and November, other types of defects that emerge seasonally may not have been captured in this