International Journal of Food Microbiology 189 (2014) 113–118

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Reduction of an *E. coli* O157:H7 and *Salmonella* composite on fresh strawberries by varying antimicrobial washes and vacuum perfusion

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ARTICLE INFO

Article history:
Received 7 April 2014
Received in revised form 18 July 2014
Accepted 2 August 2014
Available online 10 August 2014

Keywords:
Strawberry
Sanitizer
E. coli O157:H7
Salmonella
Antimicrobial

ABSTRACT

A 2011 outbreak of hemorrhagic colitis, which resulted in the death of two individuals, was associated with contaminated strawberries. A study was conducted to identify antimicrobial washes effective at reducing *E. coli* O157:H7 and *Salmonella enterica* from the surface of fresh whole strawberries during two-minute immersion washes. Twenty-seven antimicrobial treatments were tested. Vacuum perfusion was applied to strawberries during chlorine and peracetic acid treatments to promote infiltration of sanitizer into porous strawberry tissue. Strawberries were inoculated to 7.1 log CFU/strawberry with a seven-strain bacterial composite, consisting of three strains of *E. coli* O157:H7 and four serovars of *Salmonella enterica*. Berries were air-dried for 2 h and immersed in circulating antimicrobial solutions for 120 s at 22 °C. Four treatments reduced ≥3.0 log CFU/strawberry, including (a) 1% acetic acid + 1% H_2O_2, (b) 30% ethanol + 1% H_2O_2, (c) 90 ppm peracetic acid, and (d) 1% lactic acid + 1% H_2O_2. Two additional treatments that reduced 2.8 log CFU/strawberry were (a) 40% ethanol, and (b) 1% each of phosphoric + fumaric acids. Eight treatments reduced 2.0–2.6 log CFU/strawberry. Five treatments reduced <1.45 CFU/strawberry, including (a) 1% citric acid, (b) 1% lactic acid, (c) 1% acetic acid, (d) 0.5% each of acetic + citric acids and (e) 0.5% each of acetic + lactic acids. The use of vacuum perfusion with 200 ppm chlorine or 90 ppm peracetic acid did not reduce greater populations of pathogens than did the same treatments without vacuum perfusion. Fourteen treatments reduced no more pathogens ($p < 0.05$) than did sterile deionized water. Results from this study provide some options for end-point decontamination of strawberries for retail operations just prior to serving to customers.

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1. Introduction

Consumers are increasing their demand for fresh vegetables and fruits. Strawberries are esteemed as healthful and rich in vitamins and antioxidants. Some of the contributing antioxidant bioactive phytochemicals in strawberries include ellagic acid and ascorbic acids (i.e., vitamin C), and the flavonoids anthocyanin, catechin, quercetin and kaempferol. These phytochemicals are known to assist in reducing the risk of cardiovascular disease, lowering low-density lipoprotein (LDL) cholesterol (i.e., bad cholesterol), preventing thrombosis, may modulate the autoimmune inflammatory response by inhibiting COX enzymes, have anti-carcinogenic properties, and some evidence indicates that they may impede the aging process within the brain (Hannum, 2004). Nevertheless, food safety remains a problem for the consumption of fresh fruits and vegetables, including fresh strawberries.

A foodborne outbreak of *E. coli* O157:H7 in the state of Oregon in July and August 2011 was traced to contaminated fresh strawberries, which sickened 15 people, hospitalized six, and induced hemolytic uremic syndrome (HUS) in four patients. Two individuals in this outbreak, who suffered from HUS, died (Laidler et al., 2013; FDA, 2011). The implicating contaminant may have been deer feces, which tested positive for the same strain of *E. coli* as was isolated from patients. All positive environmental samples with the matching *E. coli* O157:H7 PFGE pattern contained deer feces. Another child, who lived less than 1 km away from the suspect strawberry farm, also contracted HUS from *E. coli* O157:H7. The child's clinical *E. coli* isolate proved to be a PFGE match to the strawberry farm outbreak isolates. Deer tracks were found less than 30 m from the child's house and a vacuum cleaner sample from the house tested positive for the same PFGE-matched *E. coli* O157:H7 (Laidler et al., 2013). Interestingly, a 1995 *E. coli* O157:H7 foodborne illness outbreak associated with deer jerky took place less than 60 miles away from the implicated Oregon strawberry farm (Keene et al., 1997). In addition to this *E. coli* O157:H7 outbreak, strawberries have also been implicated in outbreaks of human norovirus and hepatitis A illnesses (CDC, 1997; Niu et al., 1992; RK Institute, 2012).

Numerous publications have reported on the survival, inactivation and recovery of microorganisms on the surface of contaminated strawberries (Alexandre et al., 2012; Bialka and Demirci, 2005, 2007a,b, 2008;

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http://dx.doi.org/10.1016/j.ijfoodmicro.2014.08.005
0168-1605/Published by Elsevier B.V.

Bialka et al., 2008; Birmpa et al., 2014; El-Mogy and Alsanius, 2012; Fernández et al., 2013; Han et al., 2004a,b; Hung et al., 2010; Issa-Zacharia et al., 2010; Knudsen et al., 2001; Lukasik et al., 2001, 2003; Luksiene and Paskeviciute, 2011; Luksiene et al., 2013; Magnone et al., 2013; Raiden et al., 2003; Mahmoud et al., 2007; Rodgers et al., 2004; Tamer and Çopur, 2010; Trinetta et al., 2011, 2013; Udompijitkul et al., 2007; Yu et al., 2001; Yuk et al., 2006). Non-aqueous methods (e.g., ozone, gaseous chlorine dioxide, ultraviolet light, etc.) seem to be the preferred methods of treatment, due to the fact that aqueous washes induce fungal growth on strawberries (Yuk et al., 2006). Nevertheless, problems associated with non-aqueous decontamination of strawberries (e.g., efficacy, cost, ease of use, and worker health issues) may preclude the use of these technologies by the endpoint user in restaurants, institutional establishments and homes.

The goal of the present study, therefore, was to identify commonly-used or -reported water-soluble chemical compounds effective at reducing a seven-strain composite of *E. coli* O157:H7 and *Salmonella* from artificially-inoculated strawberries during two-minute, room temperature immersion treatments.

2. Material and methods

2.1. Bacterial strain preparation

Three strains of enterohemorrhagic *Escherichia coli* O157:H7 (EHEC) were used in this study (*E. coli* ATTC 43895, ATCC 43894, and strain C9490), as well as four serovars of *Salmonella enterica* (*Salmonella* Newport H1275 (ERRC culture collection), St. Paul 02-517-1 (cantaloupe outbreak), Stanley H0558 (CDC stool sample, 1995 sprout outbreak), and Montevideo G4639 (1993 tomato outbreak)). All isolates were selected for spontaneous mutants resistant to 100 ppm of nalidixic acid. Isolates were individually incubated for 24 h at 37 °C in 10 mL of Tryptic Soy Broth + 100 ppm nalidixic acid (TSBN), centrifuged at 4000 rpm (1980 ×g) for 10 min, and resuspended in 0.1% peptone water. All seven strains of *Salmonella* and EHEC were composited in a single sterile 500 mL beaker. The seven-strain composite was plated onto Tryptic Soy Agar with 0.1% sodium pyruvate and 100 ppm nalidixic acid (TSAPN) in order to determine the population in the inoculum. The sodium pyruvate (0.1%) was added to assist in the recovery of injured cells during subsequent antimicrobial treatments (Gurtler and Beuchat, 2005; Gurtler and Kornacki, 2009; Wesche et al., 2009).

2.2. Inoculation of strawberries

Strawberries were purchased at local supermarkets and stored at 2 °C until the time of the experiment and used within ca. 1 week of purchasing. Strawberries were equilibrated to room temperature prior to inoculation. Strawberries were immersed into the seventy-milliliter, seven-strain inoculum and then 50 mL of the inoculum was flooded over each strawberry three times to effect a 7.1 log CFU/strawberry population. Strawberries were then placed on a sterile test tube rack in a laminar flow hood and dried under continuously circulating laminar flow for 2 h at 22 ± 2 °C.

2.3. Electron microscopy

The surfaces of EHEC- and *Salmonella*-inoculated strawberries were aseptically cut into thin slices with a sterile scalpel. The thin slices were fixed for scanning electron microscopy (SEM) by immersion in a 2.5% glutaraldehyde–0.1 M imidazole buffer (Electron Microscope Sciences, Hatfield, PA) for 1 h before washing in imidazole buffer and dehydrating in 50%, 80% and absolute ethanol, successively. The samples were critical point dried (Denton Vacuum, Cherry Hill, NJ) with carbon dioxide, mounted with Duco cement (ITW Performance Polymers, Riviera Beach, FL) and colloidal silver adhesive, and sputter-coated with a thin layer of gold using a Scancoat Six Sputter Coater (BOC Edwards, Wilmington, MA). The samples were imaged with a Quanta200 FEG environmental scanning electron microscope (FEI Co., Inc., Hillsboro, OR), with an Everhart Thornley detector, operated in the high vacuum, secondary electron imaging mode at an accelerating voltage of 5 kV.

2.4. Sanitizing immersion treatments

Twenty-seven different antimicrobial washes, in addition to sterile deionized water, were tested for reducing EHEC and *Salmonella* on strawberries, as listed in Table 1. Selection of these compounds was based on their common usage by the food industry and/or reports of their use in the scientific literature. Sanitizing solutions (700 mL) were prepared in a sterile, 1000 mL beaker containing a magnetic Teflon-coated stir bar and placed on top of a magnetic stir plate. The top half of a transparent, circular polypropylene test tube rack (cut bilaterally) with holes drilled through the side wall, was placed in the beaker over a stir bar. Holes in the top of the test tube rack designed to hold 16 mm test tubes, as well as holes drilled through the sidewall permitted circulation of water throughout the beaker during treatments (Gurtler et al., 2012). An inoculated strawberry was then immersed in the sanitizing solution on top of the circular rack, while the solution was continuously agitated by the stir bar (Fig. 1). All strawberries were treated in respective sanitizing solutions for 120 s at 22 ± 2 °C. The pH of the solutions was measured using an Accumet single junction, gelled Ag/AgCl, flat surface electrode (Fisher Scientific, Pittsburgh, PA) connected to a Denver Instrument model UB-5 bench top pH meter (Denver, CO). Duplicate strawberry samples were tested in each respective solution for each experimental replication.

2.5. Strawberry sampling and enumeration of surviving bacterial cells

Strawberries were removed from treatments after 2 min, placed in a sterile, Whirl-Pak® filtered stomacher bag and diluted 1:2 (weight:volume) with Dey–Engley neutralizing broth using a Dilumat S gravimetric diluter (AES Chemunex Inc., Cranbury, NJ). Strawberries were then carefully macerated in the bags with a metal mallet and pummeled in a stomacher for 2 min. The bag-filtered homogenate was spiral plated (50 μL/plate) on TSAPN in duplicate, using a Wasp II, DonWhitley Spiral Plater (Microbiology International, Fredrick, MD). Plates were incubated for 24 h at 37 °C, and colonies were enumerated using an automated digital colony counting system (Synbiosis åCOLyte® Supercount). Presumptive-positive *E. coli* O157:H7 and *Salmonella* colonies were randomly confirmed by plating on Sorbitol MacConkey Agar (Difco), and Xylose Lysine Desoxycholate agar (Difco, Sparks, MD), respectively, and then by serological agglutination tests (Rim *E. coli* 0157:H7 Latex Test (Remel, Lenexa, KS) or *Salmonella* O Antiserum Poly A-I & Vi (Difco)). Negative control filtrates from uninoculated strawberries were also plated onto TSAPN to determine the presence of background microflora.

2.6. Vacuum perfusion treatment

To determine the efficacy of vacuum perfusion to promote infiltration of sanitizer into the contaminated strawberry surface tissues, selected treatments (peracetic acid and chlorine, as identified in Table 1) were conducted in a vacuum chamber (Bactron IV Anaerobic Chamber, Sheldon Manufacturing, Cornelius, OR). Strawberries were immersed in sanitizer solutions, and placed in a vacuum chamber on top of a battery-operated stir plate. A vacuum of 15″ Hg (50,653 Pa) was drawn in the chamber, held for 30 s, and then released to ambient air pressure for a total treatment time of 2 min (Fig. 1). A vacuum of 15″ Hg was chosen based on results from a previously published study revealing that vacuums higher than 15″ Hg resulted in low pressure-induced cracking of fruit tissue (Gurtler et al., 2012).

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