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Research note

Sanitizer applicability in a laboratory model strawberry hydrocooling system

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ABSTRACT

After harvest, strawberries are cooled to 2–5 °C, typically by forced-air cooling. Hydrocooling is an alternative method that ensures faster cooling and with the addition of various sanitizers can reduce the risk of microbial cross contamination. In this study, the effect of forced-air cooling and hydrocooling on the survival of *Lactobacillus plantarum*, a non-pathogenic, potential surrogate bacterium, was evaluated. Further, the efficacy of three antimicrobial compounds, sodium hypochlorite (HOCl, 100 μL/L), aqueous chlorine dioxide (ClO_2, 5 μL/L) and peroxyacetic acid (PAA, 80 μL/L) in reducing *Lactobacillus* levels during strawberry hydrocooling was also investigated. The results indicated that the *Lactobacillus* population on forced-air cooled strawberries was not significantly different from untreated strawberries. Compared to forced-air cooling, hydrocooling significantly reduced *Lactobacillus* survival on inoculated intact strawberries, even in the absence of a sanitizer. The addition of ClO_2 resulted in the least reduction in initial *Lactobacillus* levels (~1 log CFU/mL), similar to those on strawberries hydrocooled without antimicrobials. The addition of HOCl or PAA to the hydrocooling water resulted in respective reductions of 2.5 log CFU/mL and 4.0 CFU/mL in the initial *Lactobacillus* levels, which continued to decrease throughout the sampling period. The results from this study indicate that antimicrobials HOCl and PAA are both effective in reducing surface contamination on strawberries during hydrocooling.

1. Introduction

Strawberries are highly perishable, susceptible to moisture loss, bruising, and postharvest decay (Ferreira et al., 2008). To minimize postharvest decay, strawberries are commercially harvested at 3/4 to full-ripe stage, field-packed into retail plastic clamshells, and then into corrugated fiberboard shipping cartons. Typically strawberries are then transported to a central facility where they are rapidly cooled to temperatures ranging from 2 to 5 °C before storage and distribution (Talbot and Chau, 1991).

Forced-air cooling is used to rapidly cool strawberries, and the cooling times for the pulp temperature to reach 3 °C ranges from 60 to 90 min (Ferreira et al., 2006). Hydrocooling is an alternate method that has several advantages compared to forced-air cooling, including a faster cooling time (12–13 min), and reduced dirt/field debris, and overall microbial load (Jacomino et al., 2011; Nunes et al., 1995; Sreedharan et al., Unpublished). Strawberries are currently unwashed and field-packed for fresh market, increasing the risk of microbial contamination during cultivation, harvest and postharvest handling. Fresh and frozen strawberries have been associated with several reported foodborne illness outbreaks (FDA, 2011; Hutin et al., 1999; Niu et al., 1992). These outbreaks, along with several studies reporting prolonged survival of human pathogens, including *Salmonella* spp. and *Escherichia coli* O157:H7 on intact and cut strawberries (Flessa et al., 2005; Knudsen et al., 2001), highlight a need for better sanitation and process control.

In many operations, hydrocooling water is recirculated to conserve energy and reduce cost, which can result in the accumulation of microorganisms in the water, resulting in increased spoilage and potential foodborne illness. Addition of a sanitizer to the hydrocooling water reduces the risk of cross-contamination. Previous studies have shown that the use of various sanitizers including sodium hypochlorite (HOCl), peroxyacetic acid (PAA),

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acidified sodium chlorite (ASC), and gaseous chlorine dioxide (ClO_2) have been effective in reducing the population of human pathogens including *E. coli* O157:H7 and *Salmonella* on the surface of strawberries (Lukasik et al., 2003; Sy et al., 2005). The microbial efficacy of these sanitizers on intact strawberries during hydrocooling has not yet been evaluated.

Investigations using human pathogens cannot be conducted in food processing environments and hence a non-pathogenic surrogate organism is often used for validation studies. *Lactobacillus plantarum* has previously been evaluated as a surrogate organism for various pathogens (e.g., *E. coli* O157:H7, *Salmonella* and *Listeria monocytogenes*) (Gurtler et al., 2010; Pao and Davis, 2007; Waite-Cusic et al., 2011). Sanitizer efficacy against *Lactobacillus* during hydrocooling has not been evaluated previously. The objectives of this study were (1) compare *Lactobacillus* survival on inoculated strawberries subjected to forced-air cooling and hydrocooling, immediately after treatment, and after storage at 4 °C for 1, 3, 7 and 14 days, and (2) investigate the efficacy of selected sanitizers, HOCl (100 μL/L), aqueous ClO_2 (5 μL/L) and PAA (80 μL/L), in reducing *Lactobacillus* levels during hydrocooling of inoculated strawberries.

2. Materials and methods

2.1. Lactobacillus *strains and inoculum preparation*

L. plantarum ATCC 8014 was purchased from American Type Culture Collection, and made resistant to rifampicin (rif) as described by Kaspar and Tamplin (1993). Rifampicin resistant *Lactobacillus* cultures were then grown on MRS agar (Difco, BD, Sparks, MD) containing 100 mg/L of rif at 37 °C for 48 h. Two sequential transfers to MRS broth (containing 100 mg/L rif) were performed, followed by incubation at 37 °C. After 48 h, 10 mL of the *L. plantarum* culture was centrifuged (Sorvall RC-5B, DuPont Instruments) for 20 min at 4000 × *g*, and the resultant pellet was washed twice with buffered peptone water (BPW). The pellet was re-suspended in 10 mL BPW yielding an inoculum concentration of ca. 9 log CFU/mL.

2.2. Preparation of hydrocooling solution containing sanitizer

Forty-liter coolers (Igloo Products Corp, Katy, TX) were filled with 24 L of deionized water each and stored at 2 °C for 48 h. Sanitizer solutions were prepared using stock solutions of sodium hypochlorite (NaOCl; 5.65–6.00% Fisher Scientific, Springfield, NJ), chlorine dioxide (Aqua-Tab® tablets; BeckArt Environmental), and peroxyacetic acid (Tsunami; Ecolab, St. Paul, MN) to yield final concentrations of 100 μL/L HOCl (pH adjusted to 6.8 ± 0.05), 5 μL/L ClO_2 and 80 μL/L peroxyacetic acid (PAA), respectively. A water control with no sanitizer added to the cooler was also set up.

2.3. Inoculation, treatment and storage of intact strawberries

Fresh strawberries (*Fragaria x ananassa*; var. Radiance and Camarosa) were harvested from local farms in 454 g clamshells. Intact strawberries were spot inoculated with a single 10 μL spot of the *Lactobacillus* culture resulting in ca. 7 log CFU/berry. Inoculated strawberries were allowed to air-dry in a biosafety hood for 2 h. After drying, eight strawberries were then packed into each one-pint (473 mL) plastic clamshell (Highland Corp, Inc., Plant City, FL) and its weight recorded.

Inoculated strawberries packed in clamshells were placed on a standard strawberry cardboard flat and subjected to forced-air cooling for 90 min in a 2 °C walk-in cooler (90–95% humidity). A 94 cm long forced-air cooling tunnel, with an opening of 40.6 × 40.6 cm for loading strawberry flats was utilized for this study. Forced-air was pulled by a fan (Dayton Blower Model 4C108) driven by a 746 W motor (General Electric model 5KC48PR156U), attached to the narrow side of the tunnel 15 cm in diameter. Pressure drop across the plenum was set to 12.7 mm water column. For hydrocooling trials, 16 clamshells (each packed with inoculated strawberries) were placed in a wire basket, then immersed in coolers containing chilled water (<5 °C) with no sanitizer (control), 100 μL/L HOCl (pH 6.8), 5 μL/L ClO_2 or 80 μL/L PAA for 12 min.

After treatment, the inoculated forced-air cooled and hydrocooled strawberries were placed in an incubator (Thermo Fisher Scientific, Fairlawn, NJ) at 4 °C for 14 days, along with inoculated untreated (positive control) and uninoculated untreated (negative control) strawberries. On days 0, 3, 7 and 14, three clamshells/treatment containing strawberries were removed from the cooler for *Lactobacillus* enumeration. Each trial consisted of triplicate samples and the experiment was repeated three times ($n = 9$).

2.4. Lactobacillus *enumeration*

At each time point, five strawberries were sampled from each clamshell and placed in Whirl-Pak® bags (Fisher Scientific, Springfield, NJ), containing 100 mL of BPW and 0.1% sodium thiosulfate (Fisher Scientific, Springfield, NJ). The bags were vigorously hand shaken for 1 min, and 1 mL of the rinsate was drawn from each bag for plating. Serial dilutions were pour-plated on MRS agar plates containing rif (100 mg/L) and incubated at 37 °C for 48 h and colonies counted. The data were averaged across three replications in triplicate ($n = 3 \times 3$), and the average *Lactobacillus* CFU/mL was calculated. Analysis of variance (ANOVA) and Tukey's least square means significance test ($p \leq 0.05$) were performed on the *Lactobacillus* CFU/mL averaged across three replications in triplicate, using SAS 9.2 (SAS Institute, Cary, NC, USA).

3. Results and discussion

Lactobacillus survival on forced-air cooled fruit was not significantly different ($p > 0.05$) from that on untreated strawberries at any of the four sampling points (0, 3, 7 or 14 days post-treatment) (Fig. 1). Further, *Lactobacillus* survival on forced-air cooled strawberries was significantly higher ($p \leq 0.05$) compared to hydrocooled strawberries at all four sampling points (Fig. 1). Thus, hydrocooling significantly reduced *Lactobacillus* survival on inoculated strawberries regardless of the presence of a sanitizer.

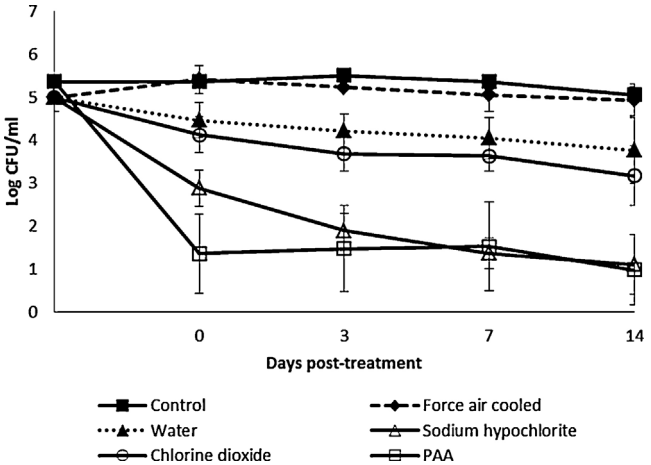

Fig. 1. *Lactobacillus* survival (log CFU/mL) 0, 3, 7 and 14 days post-treatment on intact strawberries subjected to five treatments: (■) untreated, (◆) forced-air cooled, or hydrocooled in water containing (▲) no sanitizer, (○) 5 μL/L ClO_2, (△) 100 μL/L HOCl or (□) 80 μL/L PAA. Vertical bars represent standard deviation of the mean ($n = 9$).