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Effectiveness of levulinic acid and sodium dodecyl sulfate employed as a sanitizer during harvest or packing of cantaloupes contaminated with *Salmonella* Poona

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ABSTRACT

Freshly harvested Eastern variety cantaloupes (*Cucumis melo* L. var. reticulatus cv. Athena) were subjected to three different harvest and wash treatments to examine conditions under which the efficacy of the sanitizer, levulinic acid (LV) plus sodium dodecyl sulfate (SDS), could be enhanced to reduce *Salmonella* contamination. In treatment set one, cantaloupes were spot inoculated with *Salmonella enterica* serovar Poona (prepared from solid or liquid media cultures) before or after a 1-min dip treatment in LV (2.5, 5.0, 7.5, or 10%) and 2.5% SDS. *S.* Poona initial populations on rind tissue (4.26–5.04 log CFU/sample) were reduced to detection by enrichment culture when cantaloupes were subsequently exposed to any of the LV/SDS solutions. When *S.* Poona was introduced after cantaloupes had been dip-treated, greater decreases in pathogen populations at the stem scar were observed when cantaloupes were treated with increasing concentrations of LV. In treatment set two, the response of *S.* Poona dip-treated with 5% LV/2.5% SDS was compared to a simulated commercial dump tank treatment incorporating 200 ppm chlorine as well as a two-stage treatment employing both the chlorine tank and LV/SDS dip treatments. *S.* Poona levels (log CFU/sample or # positive by enrichment culture/# analyzed) after treatments were 5.25, 3.07, 7/10, 5/10 (stem scar) and 3.90, 25/40, 28/40, 20/40 (rind) for non-treated, chlorine tank, LV/SDS dip, and tank plus dip treatments, respectively. In treatment set three, freshly harvested cantaloupes were first treated in the field using a needle-free stem scar injection (200 μl, 7.5% LV/1.0% SDS, 60 psi) and a cantaloupe spray (30 ml, 7.5% LV/0.5% SDS). Cantaloupe stem scar and rind tissue were then spot-inoculated with *S.* Poona using either a liquid or soil-based medium followed by a simulated dump tank treatment incorporating either 200 ppm chlorine or 5% LV/2% SDS. *S.* Poona inoculated on field-treated cantaloupe rind decreased by 4.7 and 5.31 (liquid) and 3.27 and 3.36 (soil) log CFU/sample after simulated chlorine and LV/SDS tank treatments, respectively. In the case of stem scar tissue, *S.* Poona populations exhibited a 1.0 log greater reduction when cantaloupes were treated with LV/SDS compared to chlorine in the dump tank ($P < 0.05$). Based on this study, application of multiple hurdles is warranted, as additional decreases in *S.* Poona populations were obtained when cantaloupes were subjected to a chlorine dump tank followed by a LV/SDS dip treatment.

1. Introduction

Salmonella spp. contamination of cantaloupes continues to be a significant concern for the produce industry. The Centers for Disease Control and Prevention has reported 9 outbreaks of salmonellosis caused by contaminated cantaloupes from 1990 to 2014 which resulted in almost 500 reported illnesses and 5 deaths (CDC, 2002, 2014). A *Salmonella* outbreak in 2012 associated with cantaloupes grown by Chamberlain Farms was significant in that 127 people were hospitalized and 3 died from consuming the contaminated fruit. As a result of these *Salmonella* outbreaks and another in 2011 involving *Listeria monocytogenes* contaminated cantaloupes (McCollum et al., 2013), several groups have created guidance documents to aid growers in the production of safe melons (Eastern Cantaloupe Growers Association, 2013; National Cantaloupe Guidance, 2014). These guidance documents recommend the use of antimicrobials in post-harvest packing processes to prevent cross contamination of wash water. Such processes could involve dump tanks, pools, flumes, bar sprayers, and hydro-coolers. Although banned in some countries, chlorine is commonly used for minimally processed produce commodities to prevent cross contamination of wash water; however, high pH and the presence of organic material in chlorine wash systems can lead to decreased effectiveness and exposure to hazardous chemicals (Gil et al., 2009; Richardson et al., 1998).

A number of sanitizers have been tested on contaminated cantaloupes as potential antimicrobials for processing. For example, chlorine (200 ppm) reduced *Salmonella* populations on cantaloupe rind by 1.8 log CFU/melon after a 60 s soak (Parnell et al., 2005). Similar decreases

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(1.5 log CFU/g) of *S.* Poona on cantaloupe rind were reported for acidified calcium sulfate (1.2%), acidified sodium chlorite (1000 ppm) and peroxyacetic acid (80 ppm) (Fan et al., 2009). Slightly greater log reductions of 2.5 and 2.3 of *Salmonella* on cantaloupe rind were obtained with lactic acid (2% for 2 min) and ozone (30 ppm for 5 min), respectively (Vadlamudi et al., 2012), whereas hydrogen peroxide (5% at 70 °C, 1 min) reduced *Salmonella* on cantaloupe rind by 3.8 log CFU/cm^2 (Ukuku et al., 2004). Chlorine dioxide (5 ppm) in both aqueous and gaseous forms decreased *S.* Poona populations by ca. 5 log CFU/g on whole cantaloupes (Rodgers et al., 2004; Mahmoud et al., 2008), but this amount exceeds the maximum level (3 ppm) allowed for application to whole produce (CFR, 2013). Hence, physical interventions have also been evaluated for their efficacy for inactivating *Salmonella* on cantaloupes. For example, hot water treatment of whole cantaloupes at 92 °C for 90 s decreased *S.* Poona levels by 5 log CFU/g on rind (Annous et al., 2013). The maintenance of high temperature wash water and the potential of contamination on the processing line after hot water treatment, however, are potential drawbacks to this type of treatment (Akins et al., 2008). Therefore, to date, most of the chemical and physical treatments do not sufficiently reduce pathogenic bacteria on cantaloupe surfaces, or they employ non-allowable or impractical procedures for the cantaloupe industry.

One chemical treatment that in recent years has shown considerable promise as an antimicrobial intervention for produce is a levulinic acid and sodium dodecyl sulfate (LV/SDS) aqueous solution. This mixture has been used to reduce pathogenic bacteria on lettuce, alfalfa seeds, and tomatoes (Zhao et al., 2009, 2010, 2011a,b). Benefits for the use of LV and SDS include their being recognized as safe food additives for specific applications by the FDA, as well as a potential for LV to be produced in large quantities at low cost (Bozell et al., 2000; Fang and Hanna, 2002). When applied to cantaloupe wash water, 2% LV and 0.2% SDS reduced *S.* Poona populations by 3.4 and 4.5 log CFU/g on netted rind tissue after a 6 min tank or tank treatment with brushing, respectively, compared to 1.6 and 2.5 log CFU/g reductions after the same process treatments with 120 ppm chlorine (Webb et al., 2013). Unfortunately, neither the 2% LV/0.2% SDS nor the 120 ppm chlorine treatment substantially reduced *S.* Poona on stem scar tissue (Webb et al., 2013).

The objectives of this study were to determine whether the efficacy of LV plus SDS for inactivating *S.* Poona could be enhanced by (1) increasing concentrations of LV plus 2.5% SDS; (2) applying LV and SDS to stem scar and rind tissue prior to their inoculation and treatment in a dump tank; and (3) incorporating dual chlorine dump tank and LV/SDS dip treatments.

2. Materials and methods

2.1. Bacterial strains

Four strains of *Salmonella enterica* serovar Poona isolated from patients who consumed contaminated cantaloupe, 01A4754, 00A3279, 01A242 and 00A3208, were obtained from Larry Beuchat at the University of Georgia, Center for Food Safety. Each strain was transformed with plasmid pDsRed-Express2 (Clonetech, Mountain View, CA) containing genes to produce *Discosoma sp.* red fluorescent protein (DsRed) and ampicillin resistance. The resulting strains produced bright red fluorescence when exposed to 500 nm using a Dark Reader trans-illuminator (Clare Chemical, Dolores, CO). The *S.* Poona pDsRed-labeled strains SD50 (01A4754), SD51 (00A3279), SD52 (01A242), and SD53 (00A3208) were stored at −80 °C in tryptic soy broth (TSB) (Neogen, Lansing, MI) with 25% glycerol. Strains from frozen stock were streaked on to tryptic soy agar (TSA) (Neogen) supplemented with 100 μg/ml ampicillin (Amp) (Thermo Fisher Scientific, Inc., Waltham, MA) and incubated at 37 °C for 18–21 h.

2.2. Cantaloupes

Freshly harvested, untreated, Eastern variety cantaloupes (*Cucumis melo* L. var. reticulatus cv. Athena) were obtained from a grower in Tifton, GA. The melons were chosen from the transport trailers to be of similar maturity, size, degree of netting, and free of any visible blemishes.

2.3. Inoculum preparation

One or two, bright red-glowing colonies from the second passage on TSA-Amp were chosen from each of the *Salmonella* strains to inoculate 50 ml of TSB-Amp (liquid media) or 3-TSA-Amp plates per strain (solid media). The liquid media culture was incubated at 37 °C with agitation (150 rpm) for 21–24 h and the solid media plates incubated at 37 °C for 24 h. Colonies were gently removed from solid media plates with 4 ml of 0.1% peptone water (Difco, Sparks, MD) by a glass spreader and collected in a 50-ml centrifuge tube. Remaining colonies were dislodged from plates with an additional 4 ml of 0.1% peptone water. Both types of cultures were individually sedimented by centrifugation (4193 ×*g* for 15 min at 4 °C), washed two times in sterile 0.1% peptone water, and resuspended in 3 ml of 0.1% peptone water. Individual strains from each preparation were combined in equal proportions to make a mixture of ca. 10 log CFU/ml. Preliminary cultivation trials had been performed to confirm that this high concentration of *S.* Poona could be achieved from both solid and liquid media-grown cultures of all four isolates. In addition, the stock cultures were enumerated to confirm the target population in each replicate trial. Liquid and solid media-grown stocks were diluted in 0.1% peptone water to 8 and 9 log CFU/ml for spot inoculation of stem scar and rind tissue, respectively.

Soil was collected from the cantaloupe farm and sifted to remove rocks and other large debris. A portion (200 g) was placed in a 3.07-l Glad container (The Clorox Company, Oakland, CA) and 4 ml of a 10 log CFU/ml *S.* Poona pDsRed mixture was applied in a fine mist spray. The inoculated soil was mixed for 1 min with a spoon, covered, and held overnight in the dark for pathogen acclimation.

2.4. Preparation of sanitizing solutions

Levulinic acid (98%, Acros Organics, Fair Lawn, NJ) and sodium dodecyl sulfate (20%, Acros Organics) were combined with sterile deionized water to make dip treatments comprised of either 3 or 4 l of 2.5, 5.0, 7.5, or 10.0% LV and 2.5% SDS with pH values of 3.1, 2.9, 2.7, and 2.7, respectively. Solutions were also made for cantaloupe spray (2-l, 7.5% LV/0.5% SDS, pH 2.7), stem scar injection (200 ml, 7.5% LV/0.5% SDS, pH 2.71), and dump tank (10-l 5% LV/2.0% SDS, pH 2.8) treatments. The dip, spray, stem scar injection, and dump tank solutions of LV/SDS were prepared fresh for each replicate trial. Chlorine (200 ppm) was prepared by adding approximately 40 ml of sodium hypochlorite solution containing 5% available chlorine (Ricca Chemical Company, Arlington, TX) to 10-l of sterile de-ionized water. The chlorine solution was adjusted to pH 7.0 with sulfuric acid (Sigma-Aldrich, St. Louis, MO). Free chlorine was determined by the Hach digital titrator using the DPD-ferrous ethylenediammonium sulfate titration cartridge (Hach Co., Loveland, CO). Chlorine solutions were prepared fresh for each replicate trial.

2.5. Treatment of cantaloupes by dip treatment with different concentrations of levulinic acid and 2.5% SDS

Cantaloupes held at room temperature (~22 °C) for 5 or 9 h (replicates 1 and 4, respectively), 24 h (replicate 2), or 48 h (replicate 3) after harvest were spot-inoculated either prior to or after sanitizer treatments. In either case, 10 μl of a 9-log CFU of *S.* Poona pDsRed/ml inoculum or 10 μl of a 8-log CFU/ml inoculum, prepared from cultures grown either in liquid or on solid media, was applied within 2-cm diameter templates marked on netted rind or to stem scar tissue, respectively. Melons inoculated pre-treatment were held for 18 h at 22 °C. Cantaloupes that were dip-treated involved submersion for 1 min in 11.35-l pails (33.02 cm diameter, 26.04 cm height; Walmart, Bentonville, AR) containing 3-l of sanitizer solution (2.5, 5.0, 7.5, or 10% LV and 2.5% SDS). After treatment, melons were placed in clean 354-ml foam bowls (Walmart, Bentonville, AR)