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Using antimicrobials as a food safety measure during phytosanitary treatments in mangoes

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ABSTRACT

Prevention of plant-borne pest infestation necessitates use of phytosanitary procedures, as in the case of U.S.-imported mangoes. Supplementation of hydrothermal disinfestation and/or post-process cooling waters with chemical sanitizers could provide mango packers with antimicrobial interventions reduce or prevent microbial pathogen transmission on mangoes. The current study determined: i) the effectiveness of chlorine (CL) or lactic acid (LA) addition to water used for hydrothermal and cooling treatments to reduce *Salmonella* survival on mangoes during disinfestation treatment, and; ii) *Salmonella* internalization into stem scars following hydrothermal and cooling treatments in sanitizer-supplemented water. *Salmonella* survival during post-treatment storage and effects of treatments on mango color and firmness were also determined. A 2.0 log-cycle reduction was obtained on stem scars subjected to hydrothermal treatment; reductions of 2.2 and 1.3 log-cycles were obtained on stem scars with LA and OCl^- treatment, respectively. An additional 1.0 log-cycle reduction during cooling was observed for OCl^--treated mangos; *Salmonella* were not detected (< 2.0 log CFU/10 cm^2) on LA-treated mangos. On hydrothermal-treated rinds, a 0.5 log cycle reduction was obtained for control fruit; a reduction of 1.7 log cycles was obtained for both LA- and OCl^--treated fruit. Internalized *Salmonella* were detected in stem scar tissues obtained following hydrothermal treatment and cooling by enrichment, and survived storage at 10 °C for 12 days. In general, there were no differences in the reduction of *Salmonella* between CL and LA, although in two occasions CL was less effective. However, mango color was compromised by use of LA. Chlorine use both in hot and cool dips is recommended for minimizing *Salmonella* transmission on mango surfaces.

1. Introduction

Increasing awareness of health benefits coupled with advancements in processing, packing, and preservation technologies has resulted in increased consumption of fresh produce among high income population in the U.S. and in an increase in the imports of these commodities to supply a year round demand (Pollack, 2001). Concurrently, an increasing number of fresh produce-transmitted foodborne disease outbreaks have been reported by the Centers for Disease Control and Prevention (CDC) in recent years as estimated by Painter et al. (2013), reporting that estimated 46% of foodborne disease outbreak cases were attributable to plant-derived foods. Also, *Salmonella* was reported to be the causative agent of the greatest number of bacterial foodborne disease cases especially with produce related outbreaks and involving domestically grown as well as imported and imported fresh fruits and vegetables (Scallan et al., 2011).

One of the major concerns about importing produce to the U.S., is the possible introduction of pests. To protect against invasive insect species being imported, mangoes are commonly subjected to disinfestation treatments to comply with regulatory demands in the U.S. and abroad (Jacobi et al., 2001). Regulations of the U.S. Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS)

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require that specific produce commodities be subjected to validated disinfestation treatment before allowing entry to the U.S. (Title 7, U.S. Code of Federal Regulations §305). Compliance with this rule is commonly achieved by subjecting the product such as foreign mangoes, to immersion in warm water (also known as hydrothermal treatment) for a sufficient time to reach an internal temperature of 46–47.8 °C (APHIS, 2016), usually between 90 and 120 min depending on the size of the mango. Although not required by APHIS, the industry may choose to apply a cooling step to prevent quality loss. This is commonly achieved by hydrocooling, dipping in chlorinated water. In addition to chlorine, additional chemicals may be used if approved by the Food and Drug Administration (APHIS, 2016). The thermal gradient between the cooling water and the warm mango flesh may result in a pressure difference that may force the entry of water to the warm mango. If the water is contaminated with microorganisms, the mango may be internalized by the microorganisms (Bartz and Showalter, 1981). In the U. S., three outbreaks of *Salmonella* infection have been linked to imported mangoes. In December 1999, an outbreak of *Salmonella enterica* subsp. *enterica* serovar Newport infections involving Brazil-grown mangoes produced 78 cases of human disease, with 2 fatalities (Sivapalasingam et al., 2003). Use of *Salmonella*-contaminated water for disinfestation process of mangoes, along with lack of chlorination, was suggested as the cause for the cross-contamination of mangoes, leading to the outbreak (Sivapalasingam et al., 2003). A second outbreak involving 26 *Salmonella* infections occurred in 2001 (Beatty et al., 2004). This outbreak was caused by *Salmonella* Saintpaul and was linked to the consumption of raw mangoes imported from Peru. Investigators suspected that, similar to the 1999 *S.* Newport outbreak, mangoes were cross-contaminated during disinfestation treatment by treatment waters that were not adequately chlorinated (Beatty et al., 2004). The potential for *Salmonella* to internalize into mangoes during elevated heat exposure followed by cooling, was tested and verified by Penteado et al. (2004) on Florida-grown Tommy Atkins mangoes and later by Bordini et al. (2007) on Brazil-grown Tommy Atkins mangoes. Another outbreak of human disease linked to consumption of contaminated mangoes occurred in 2012 and was caused by *Salmonella* Braenderup present in Mexico-exported mangoes. This incident involved 127 cases with 33 hospitalizations across 15 states (CDC, 2012).

After repeated studies indicating that a pressure differential can lead to internalization of mangoes by bacterial pathogens (Bordini et al., 2007; Penteado et al., 2004) and the observations during field investigation during the outbreaks linked to mangoes imported from Brazil and Peru (Beatty et al., 2004; Sivapalasingam et al., 2003), attention has been called to the need for mango producers to utilize process water decontamination interventions to prevent enteric foodborne pathogens from contaminating mangoes during disinfestation treatment prior to cooling and shipping. Water disinfection is often used in the produce industry to prevent cross contamination during tank washing as well as during hydrothermal treatment of mangoes, chlorine being the most popular compound for this purpose. However, some groups tend to express concerns about the safety of chlorine (Bull et al., 1995). Natural antimicrobials, such as lactic acid, may be a good alternative to chlorine in water disinfection especially used for washing procedure, as previously reported (Ibarra-Sánchez et al., 2004). Thus, further research is needed to assist mango producers in maintaining microbial food safety while implementing mandated phytosanitary quarantine techniques that effectively decontaminate mango surfaces while not allowing for pathogen internalization. Additionally, such processes should not result in significant loss of mango quality. The current study aimed at understanding the effect of addition of chlorine or lactic acid to water used for hydrothermal and cooling treatments on the survival of *Salmonella enterica* serovars on the rind and stem portions of inoculated mangoes. The effect of various antimicrobials added to the water used for both hydrothermal and cooling treatments on the internalization of the pathogen in the stem tissue. Finally, impacts of hydrothermal treatments on mango physico-chemical attributes (firmness, color) were determined.

2. Materials and methods

2.1. Collection of mangoes

To ensure use of foreign-grown mangoes without being subjected to quarantine treatment, all experiments were conducted in the Food Microbiology and Safety Laboratory at the University of Guadalajara's University Center for Exact Sciences and Engineering (CUCEI). Fresh mangoes (*Mangifera indica*) grown in Jalisco State were obtained from a distributor in Guadalajara, Mexico, ensuring all mangoes were of the same origin. Mangoes were not waxed, not hydrothermally or otherwise treated, were free of visual defects such as bruises, cuts or abrasions, and were of similar size and maturity (mature green). In three separate occasions, a batch composed of 630 units was brought to the laboratory for experimental studies on the same day of collection. Each time, the mangoes were separated into 9 groups of 70 units, and each group was subsequently separated in two sub-groups of 35 units. Both subgroups were inoculated as described in 2.3 and then subjected to the same treatments as described in 2.4. Thirty mangoes of one subgroup were used for studying the effect of hydrothermal and cooling steps on the potential for cross contamination of *Salmonella*, and 30 in the other group were used for determining the survival of *Salmonella* on and inside mangoes during storage. The remaining 5 mangoes on each subgroup were used to determine the potential for internalization of *Salmonella* after exposure of hot mangoes after hydrothermal, to cooling.

For separate experiments determining the effect of the treatments on the quality of the mangoes, 150 mangoes were obtained in 3 occasions from the same sources, selecting them to have similar size, visual color and maturity stage as the batches used for the inoculated experiments. These mangoes were not inoculated and were used for instrumental analysis for color and firmness, as described in Section 2.7.

2.2. Bacterial inoculum preparation and mango inoculation

Strains of *Salmonella enterica* serotypes Poona, Montevideo, Agona, Michigan, Newport, and Gaminara were obtained from the Texas A&M University Food Microbiology Laboratory culture collection. Rifampicin-resistant (Rif^+) variants of these strains were used to differentiate the inoculated cells from other salmonellae potentially present in the mangoes. Preliminary studies demonstrated that the Rif^+ variants followed the same behavior as their parent strains. The strains were stored in freezing beads (Cryocare, Key Scientific Products, Round Rock, Texas) at −80 °C until activated for use. The microorganisms were resuscitated by two successive transfers into Tryptic Soy Broth (TSB; Becton, Dickinson and Co., Sparks, MD) and incubated at 35 °C for 18–24 h. The cells were then transferred onto Tryptic Soy Agar (TSA; Becton, Dickinson and Co.) slants and stored at 4–5 °C until they were needed for the experiment. Prior to use, resistance to rifampicin was confirmed by streaking each strain onto TSA plates supplemented with 80 μg/mL rifampicin (Sigma-Aldrich Co., St Louis, MO) and incubated at 35 °C for 18–24 h. Colonies were then maintained on TSA slants and transferred twice to TSB and incubated at 35 °C for 18–24 h for inoculum preparation.

The day before experiment initiation, each isolate was transferred into a glass bottle (six bottles in total) containing 250 mL TSB and incubated at 35 °C for 18–24 h. After overnight growth, bottles contained 8.0–9.0 log CFU/mL of corresponding *Salmonella* strains. Cells were then harvested by centrifuging ($1610 \times g$) for 10 min at 4 °C. The cell pellets obtained were re-suspended individually in sterile bottles containing 250 mL 0.1% sterile peptone water. The bottles containing each suspended strain contained in individual bottles (250 mL each for a total of 1.5 L) were transferred into a sterile plastic bucket containing 2.5 L 0.1% sterile peptone water, preparing the 4 L of cocktail. Prior to