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Tomato type and post-treatment water rinse affect efficacy of acid washes against *Salmonella enterica* inoculated on stem scars of tomatoes and product quality☆

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ABSTRACT

A study was conducted to evaluate the effects of post-treatment rinsing with water on the inactivation efficacy of acid treatments against *Salmonella* inoculated onto stem scar areas of two types of tomatoes. In addition, impact on fruit quality was investigated during 21 days post-treatment storage at 10 °C. A four-strain cocktail of *Salmonella enterica* (*S*. Montevideo, *S*. Newport, *S*. Saintpaul, and *S*. Typhimurium) was inoculated onto stem scar areas of grape and large round tomatoes. The inoculated fruits were then treated for 2 min with the following solutions: water, 2% lactic acid + 2% acetic acid + 2% levulinic acid, 1.7% lactic acid + 1.7% acetic acid + 1.7% levulinic acid, and 3% lactic acid + 3% acetic acid. After treatments, half of the fruits were rinsed with water while another half were not rinsed. Non-inoculated grape tomatoes for quality analysis were treated with the same solutions with and without subsequent water rinse. Results demonstrated that the acid combinations reduced populations of *Salmonella enterica* on the stem scar area of grape tomatoes by 1.52–1.90 log CFU/fruit, compared with the non-treated control while water wash and rinse removed the bacterium by only 0.23–0.30 log CFU/fruit. On the stem scar of large round tomatoes, the same acid treatments achieved 3.54 log CFU/fruit reduction of the pathogen. The varying response to the acid washes between grape and large round tomatoes seems to be related to the differences in surface characteristics of stem scar areas observed with SEM. Rinsing with water after acid combination treatments did not significantly affect the efficacy of the treatments in either grape or large round tomatoes. Acidic off-odor was detected on fruits treated with acid combination without water rinse 1 day after treatment while water rinse eliminated the off-odor. The acid treatments with and without water rinse did not consistently affect appearance, color, firmness, or lycopene or ascorbic acid contents of tomatoes during 21-days storage at 10 °C. Considering the similarity in antimicrobial efficacy between the fruits with and without water rinse following acid treatments, and the elimination of acidic odor by water rinse, fruits should be rinsed with water after acid treatments. Overall, our results demonstrated that the acids were more effective in inactivating *Salmonella* on large round tomatoes than on grape tomatoes, and water rinses following acid treatments eliminated the acidic odor without affecting the efficacy of the acids against *Salmonella*.

1. Introduction

Fresh tomatoes are a popular commodity in the U.S. and around the world. However, consumption of raw tomatoes has been implicated in at least 15 multistate *Salmonella* outbreaks, resulting in 1959 illnesses, 384 hospitalizations, and three deaths in the U.S. in recent years (Bennett et al. 2015). The industry relies primarily on washes with sanitizers, such as chlorine, to minimize cross contamination. However, chlorine has limited efficacy against human pathogens on tomatoes, especially when pathogens reside in stem scar areas (Yuk et al., 2005).

Many intervention technologies other than chlorine have been studied, most of which have limited effectiveness, likely due to the presence of pathogens in the protective sites such as stem scar areas (Bartz et al. 2015). Recent research has focused on natural antimicrobials in light of consumers' decreasing preference of artificial or synthetic additives. Organic acids such as acetic, citric, malic, lactic, and levulinic acids have been evaluated as alternatives to synthetic antimicrobials such as chlorine (Gil et al. 2009; Park et al. 2011). Organic acids are

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natural substances found in various fruits and vegetables and exhibit antimicrobial activity against foodborne pathogens (Doores 2005) and are "generally recognized as safe" (USFDA 2017b). It is believed that organic acids inactivate microorganisms via a number of mechanisms. The main mechanism involves undissociated organic acids that penetrate the cell membrane of bacteria. The acidic pH inside the cell causes damage to enzymes, proteins and DNA structure (Mani-Lopez et al. 2012).

In et al. (In et al. 2013) compared various organic acids against *Shigella* and found that acetic acid exhibited the greatest antimicrobial activity in the paper disk diffusion experiment, but lactic acid was the most effective antimicrobial agent against *Shigella* species artificially inoculated onto lettuce. Antimicrobial activity of organic acids (lactic, citric, acetic, and ascorbic acid) against *E. coli* and L. *monocytogenes* was compared on Iceberg lettuce (Akbas and Ölmez 2007). Dipping Iceberg lettuce in 0.5% citric acid or 0.5% lactic acid solutions for 2 min was as effective as chlorine for reducing microbial populations.

Levulinic acid has shown considerable promise as an antimicrobial intervention for fresh produce in recent years, particularly when used in combination with sodium dodecyl sulfate (SDS) (Zhao et al. 2009, 2010). The combination has been used to reduce pathogenic bacteria on lettuce and cantaloupe (Guan et al. 2010; Webb et al. 2015; Zhao et al. 2009). The combination of 2% levulinic acid and 0.2% SDS reduced *S.* Poona populations by 3.4 log CFU/g on netted rind of cantaloupe after a 6 min tank treatment (Webb et al. 2015). In contrast, only 2.6 and 2.5 log reductions of *Salmonella* were inactivated on stem scars of tomatoes and cantaloupes, respectively, after treatment with 2% levulinic acid plus SDS, suggesting the challenge in inactivating pathogen on scar tissues.

To increase the efficacy of organic acids against human pathogens, organic acids have been combined with many other sanitizers and technologies such as essential oils, ozone, ClO_2, ultrasound, and other organic acids for different types of produce (de São José et al. 2014; Nazer et al. 2005; Yuk et al., 2005; Yuk et al. 2006; Zhou et al. 2007). Gurtler et al. (2012) tested the combinations of many different acids against *Salmonella enterica* inoculated onto the stem scar of red round tomatoes. Solutions that achieved ≥4.95 log reductions were 1.7% lactic acid +1.7% acetic acid +1.7% levulinic acid and 3% lactic acid +3% acetic acid. However, the fruits were not rinsed with water after treatments. It is known that rinsing with water after sanitization treatment reduces efficacy of sanitizers (Sapers et al. 2000) as rinsing removed residual acid on the fruit. Many acids, such as acetic acid and peroxyacetic acid have a pungent odor. The odor may limit their use on fresh produce. It has been found that Iceberg lettuce and parsley treated with vinegar (containing > 2% acetic acid) developed strong, unacceptable odor and flavor (Chang and Fang 2007; Vijayakumar and Wolf-Hall 2002; Wu et al. 2000). It is unclear whether the combined acid treatments would result in development of an acetic odor on tomatoes. Rinsing with water after acid washes would be expected to reduce the acidic odor. In addition, the effectiveness of acid combinations have not been evaluated for grape tomatoes, which are more often consumed raw in salads. Furthermore, the effects of the acid combinations on other quality attributes of tomatoes have not been studied either. Therefore, the objectives of the present study were to evaluate the efficacy of washing with acid combinations against *Salmonella enterica* on grape tomatoes, and to evaluate the effects of post acid treatment rinsing with water on the antimicrobial efficacy and impact of acid wash and water rinsing on fruit quality during 21 days of storage.

2. Materials and methods

2.1. Sources of bacteria strains and organic acids

The following four pathogenic *Salmonella enterica* strains were used in the study: *Salmonella* Montevideo (*Salmonella* group C, ATCC #8387), *Salmonella* Typhimurium (group B, ATCC #14028) *Salmonella* Newport (group C, Eastern Regional Research Center [ERRC] culture collection), and *Salmonella* Saintpaul (group B, isolate #02-517-1 from a cantaloupe outbreak isolate via Bassam Annous, ERRC). Acetic acid (99.8–100.5% purity) and levulinic acid (98% purity) were purchased from Sigma-Aldrich (St. Louis, MO) while lactic acid (85% purity) was from Spectrum Chemical (Gardena, CA).

2.2. Inoculation and treatments of tomatoes

Each strain of *Salmonella enterica* was selected for nalidixic acid-resistance by successive growth of each strain in tryptic soy broth (TSB) with increasing nalidixic acid concentrations to 100 μg/g (ppm) (Fan et al. 2012). Each isolate was then incubated in 10 ml TSB with 100 μg/g nalidixic acid (TSBN) for 24 h at 37 °C. The cultures were centrifuged for 10 min at 2812 ×*g*, re-suspended to the original suspension volume with sterile 0.1% peptone water, and composited in one single inoculum with a total volume of 40 ml.

Grape tomatoes and large round tomatoes were purchased from local supermarkets (Philadelphia, PA). To inoculate the stem scar area of grape tomatoes, 25 μl inoculum was deposited, with a micropipetter, onto the stem scar of each fruit which set on a rack. Ten fruits were inoculated for each treatment per trial. Fruits were then air dried, with the stem side up, in a laminar flow hood for 2 h. Ten tomatoes were then treated as described earlier (Gurtler et al. 2012) with the following solutions (400 ml): water, 2% lactic acid +2% acetic acid +2% levulinic acid, 1.7% lactic acid +1.7% acetic acid +1.7% levulinic acid, and 3% lactic acid +3% acetic acid. Grape tomatoes were placed in 400 ml solutions in a 1 l beaker containing a stir bar. A circular polypropylene test tube rack with holes drilled through the side wall was placed in the beaker over a stir bar to avoid direct contact of stir bar with fruit. The solution was continuously agitated by the stir bar for 2 min. Following treatment, half of the tomatoes (5) were immediately rinsed in deionized water for 1 min (in 400 ml water) using the same procedure for the acid treatments, while the other half of the fruit was not rinsed. For experiments on large round tomatoes, fruits were handled similarly as for grape tomatoes with the exception that each fruit was treated separately after inoculation with 100 μl inoculum onto stem scar areas. To directly compare the efficacy of acid combinations in inactivating *Salmonella* on grape and large round tomatoes, the following two acid combinations (2% lactic acid +2% acetic acid +2% levulinic acid, and 3% lactic acid +3% acetic acid) were applied to the two types of fruits with and without subsequent water rinse.

2.3. Recovery of Salmonella from tomatoes

Following treatments, stem scars were removed from each fruit using a pair of sterile scissors. The stem scars of 5 grape tomatoes were combined and weighed, and then placed in up to 15 ml (10 times of sample weight) of buffered peptone water (Difco Laboratories, Becton, Dickinson & Company, Sparks, MD) in an 80 ml stomacher bag and pummeled in a filtered stomacher bag for 2 min at 260 rpm. For large round tomatoes, stem scars were not combined. Each individual stem scar was stomached in up to 15 ml of buffered peptone water for 2 min. Sample filtrate after proper dilution with phosphate buffer saline (PBS) was plated on TSA with 100 μg/g nalidixic acid and 0.1% sodium pyruvate. All plates were incubated for 24 h at 37 °C before colonies were counted.

2.4. SEM

Stem scars of non-inoculated grape and large round tomatoes were excised using a pair of sterilized scissors and placed into 5 ml of 2.5% gluteraldehyde [Electron Microscopy Sciences (EMS), Hatfield PA, USA] and were allowed to fix for 1 h. The stem scar samples were then rinsed with 0.1 M imidazole buffer (EMS), and dehydrated in 2 ml