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Inactivation of *Salmonella* in grape tomato stem scars by organic acid wash and chitosan-allyl isothiocyanate coating☆

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ABSTRACT

The objective of this study was to evaluate inactivation of inoculated *Salmonella enterica* on grape tomato stem scars exploiting integrated treatment of organic acid wash (AW) followed by chitosan-allyl isothiocyanate (CT-AIT) coating. The treatment effect on microbial loads and fruit quality during 21 days storage at 10 °C was also determined. A bacterial cocktail containing three serotypes of *Salmonella enterica* was used for this study based on their association with produce-related outbreaks. Tomatoes were spot inoculated on stem scars and then immersed in an organic acid solution (700 ml) containing 0.5% (*v*/v) each of acetic (AA) and formic acid (FA) to wash under mild agitation for 1 min at ambient temperature (22 °C) followed by 1 min dipping in a coating solution containing 6 ml AIT/g CT. AW in 0.5% organic acid (AA + FA) for 1 min reduced *Salmonella* population by 2.7 log CFU/g from an initial load of 7.8 log CFU/g, while additional coating treatment of AW tomatoes reduced the pathogens on stem scars to undetectable levels (< 0.7log CFU/g), achieving, in combination, a > 7 log CFU/g reduction for the pathogen. Although the populations of *Salmonella* in the controls (approx. 7.8 log CFU/g stem scar) did not change significantly during 21 days of storage at 10 °C, the populations were reduced to undetectable level in the integrated (AW plus CT-AIT) treated stem scars on day 1 and no regrowth was observed during storage. The treatment significantly ($p < 0.05$) reduced background bacterial loads to approx. 1.3 log CFU/g and the population remained unchanged through day 21 at 10 °C. The treatment also completely inactivated mold and yeast on day 1 with no growth reoccurrence. These results indicate that the integrated treatment can provide a safe and effective intervention strategy for grape tomatoes.

1. Introduction

The microbial safety of fresh fruits and vegetables continues to be a major concern. Tomatoes, leafy greens, and melons have been frequently implicated in outbreaks of foodborne illness accounting for approximately two-thirds of all produce related outbreaks. Between 1996 and 2006, tomatoes were associated with several multi-state outbreaks, accounting for 17% of all produce related outbreaks (Gravani, 2009). The most frequently identified and reported bacterial pathogen was *Salmonella* which has been linked to multiple confirmed cases of salmonellosis since 1990 (CDC (Centers for Disease Control and Prevention), 2017; Bidol et al., 2007).Epidemiological studies indicate that contamination of tomatoes with pathogens can occur anywhere during production and processing (Hanning et al., 2009). *Salmonella* may internalize through the wounds on the surface or through the stem or blossom scar into tomatoes. Stem scars appear to provide greater possibility for survival and/or growth of pathogens and hence more difficult to inactivate without creating adverse effects on sensory quality (Yuk et al., 2005). Once internalized, *Salmonella* can survive in the low pH of tomato fruit, as low as pH 4.0 (Asplund and Nurmi, 1995) and grow throughout the normal shelf life period (Beuchat and Mann, 2008).Sanitizer wash is the principal processing step followed in the produce industry to reduce cross contamination.

The stem scar region of tomato has been indicated as the potential

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source for enteric pathogen contamination. Unlike the smooth surface, stem scar areas are highly porous in nature (Guo et al., 2002) which creates greater obstacle for the sanitizer molecule to reach to the pathogens in the pores of stem scars limiting the effectiveness of sanitizer wash (Guo et al., 2002; Mukhopadhyay et al., 2014). Iturriaga and Escartín (2010) reported the formation of biofilm on tomato cuticles. *Salmonella* is capable of forming and adhere to biofilm (Yaron and Romling, 2014). Hence, in addition to resistance to sanitizer molecule transport to stem scars, the formation of biofilm can also lead to persistence and resistance to disinfection treatments.

Currently, the produce industry relies on a chlorine based sanitizer wash to reduce the risk of cross contamination where tomatoes are dumped into flume tanks containing up to 200 ppm free chlorine for a short duration before packing (Bartz et al., 2001). However, effectiveness of chlorine wash is limited to about 2 log CFU reductions of pathogens (Brackett, 1999; Herdt and Feng, 2009; Yuk et al., 2005). Studies indicate that washing tomatoes in 100 ppm (Wei et al., 1995) and 320 ppm (Zhuang et al., 1995) free chlorine for 2 min cannot completely inactivate *Salmonella*, implying that more effective means of sanitizer treatments are needed for tomato fruits. Also, the effectiveness of chlorine reduces rapidly on contact with organic matter (Solomon et al., 2002) and chlorine reacts with organic materials in wash water to form organochlorine byproducts, a suspected carcinogen (Richardson et al., 1998) which may impose new regulatory restrictions (Allende et al., 2004: Artes et al., 2009). Due to the severity of this problem there is a need for development of a safe and effective intervention strategy in addition to current chlorine-based wash (Ibarra-Sanchez et al., 2004).

During the past decade, a number of nonthermal methods have been proposed to inactivate *Salmonella* on produce. These include organic acid wash (Li and Wu, 2013; Mukhopadhyay et al., 2015: Neal et al., 2012), antimicrobial coating (Jin and Niemira, 2011; Park and Zhao, 2004) and other physical or chemical interventions. Organic acids are 'generally recognized as safe' (GRAS), economic and easy to manipulate. Organic acids have shown to prevent bacterial growth effectively (Mani-Lopez et al., 2012; Taylor et al., 2012). Chitosan which is a deacetylated derivative of chitinan and the byproduct of seafood processing has a broad antimicrobial activity (Helander et al., 2001) and film-forming properties (Olmez and Kretzschmar, 2009). Antimicrobials can be incorporated in chitosan-based films and released in a controlled manner (Park and Zhao, 2004). Chitosan based antimicrobial formulation is suitable for coating application for produce.

Allyl isothiocyanate (AIT) is a GRAS natural food preservative which belongs to plant Cruciferae family. AIT has been shown to have strong antimicrobial activity in liquid media as well as in its vapor form (Lin et al., 2000). Authors in this work investigated the mechanism of its antimicrobial activity and reported the mechanism of AIT on cell membranes and on leakage of cellular metabolites being similar to polymyxin B, an antibiotic of known antibacterial mechanisms Chitosan based AIT has been used in coating application for inactivation of *Salmonella* on produce (Duan et al., 2007; Zivanovic et al., 2005) and many other food-borne bacteria. Jin and Gurtler (2012) studied antimicrobial effect of various chitosan based antimicrobial coatings on tomatoes and reported 6 log CFU reduction of *Salmonella*. Report on chitosan based coatings with 60 μl/ml AIT indicated > 5 log CFU/cm^2 reduction of *Salmonella* inoculated on fresh cantaloupe surface. Inactivation efficacy of the pathogen increased with higher concentration of AIT in the coating (Chen et al., 2012). These nonthermal methods have the ability to inactivate microorganisms to varying degrees depending on the type of pathogen, produce and the nature of contaminated surface. Reports on *Salmonella* decontamination within the stem scar region of tomatoes and associated quality effects are scant. Pathogens in stem scars require stringent treatment (Yuk et al., 2005). However, if the treatment intensities are too high, as often is the case with a single intervention strategy, it can cause adverse effects on produce sensory quality (Wei et al., 1995). Integrated or combined treatment method have the ability to inactivate pathogens to the recommended level with minimal quality effect at a lower intensity of individual treatments compared to a single treatment strategy (Mukhopadhyay and Gorris, 2014a).

Previously inactivation of *Salmonella* on whole plum tomato surface by integrated treatment strategy was reported (Mukhopadhyay et al., 2015). The aim of the present study was to evaluate the antimicrobial efficacy of an integrated method of organic acid wash and chitosan-AIT post-wash coating against *Salmonella* on stem scars of grape tomatoes. The other objectives were to examine the effects of the combined treatment on the native microbial populations and the sensory quality of grape tomatoes during storage. The final goal of this study was to develop an effective non-chlorine based intervention method that would improve the safety and shelf-life of tomatoes.

2. Materials and methods

2.1. Tomatoes (Solanum lycopersicum)

Whole grape tomatoes (species *S. lycopersicum*, variety Santa), fresh and unblemished were purchased from a local super market (Wyndmoor, PA). Tomatoes were purchased on the day before experiment and kept in the refrigerator overnight at 4 °C. On the day of experiment, tomatoes were taken out of the refrigerator and placed inside hood for 2 h to acclimatize to room temperature before chlorine wash and inoculation. Ninety six fruits were washed with 200 ppm of chlorine (pH 6.5) for 2 min to reduce natural microbiota and minimize antagonistic effect of natural microbiota to the inoculated pathogens, since report indicate possible antagonistic effect natural microflora to inoculated pathogen on produce surface (Ukuku et al., 2004). Clorox, a commercial bleach containing 5.25% sodium hypochlorite (NaOCl, Clorox Company, Oakland CA), was diluted with sterile water to provide a concentration of 200 mg/L of available chlorine in the wash solution. Free chlorine in the solution was determined with a chlorine test kit (Hach Co., Ames, IA) that has been approved by the U.S. Environmental Protection Agency. The pH was adjusted to 6.4 ± 0.1 by adding citric acid. After wash, fruits were rinsed with water, and dried on paper towels for 2 to 3 h in a laminar hood at ambient temperature before being inoculated. However, a set of tomatoes were not washed to remove background microorganisms prior to use in experiments to determine the background microbial loads.

2.2. Strains, growth conditions, and inoculum preparation

A bacterial cocktail composed of three serotypes of *S.* enterica (*S.* Montevideo G4639, *S.* Newport H1275, and *S.* Stanley H0558) was used for this work. Selection of these strains was based on their association with fresh produce related outbreaks. *S.* Montevideo G4639, which was isolated from a tomato-associated outbreak, was received from Dr. Larry Beuchat at the University of Georgia. *S.* Newport H1275 and *S.* Stanley H0558 both were associated with alfalfa sprout-related outbreaks and were obtained from Dr. Patricia Griffin, Center for Disease Control and Prevention, Atlanta, GA. Individual strain were grown in 5 ml Tryptic soy broth (TSB, Difco, BD) incubated in static at 37 °C for 24 h. After 24 h of incubation, a loop transfer of these strains were made into new 5 ml Tryptic soy broth and were similarly incubated at 37 °C for 24 h. A final transfer of 0.2 ml was made into 50 ml TSB with incubation at 37 °C for 18 h. The bacterial cells were harvested by centrifugation (5000 × *g*, 15 min) at 4 °C. Cell pellets were washed twice in 0.1% (*w/v*) peptone water (PW, BBL, BD Difco) and was finally suspended in PW to a achieve target level of about 8 log CFU/ml. To enumerate the population densities in each cell suspension, appropriate dilutions (in 0.1% PW) were spiral plated, in duplicate, on to tryptic soy agar (TSA; BD Difco) plates. Equal volumes of cultures were combined in a separate sterile test tube to obtain a cocktail of three strains of *Salmonella* prior to inoculation of tomatoes.