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Inactivation of *Salmonella* spp. on tomatoes by plant molecules

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ABSTRACT

The efficacy of carvacrol (CAR), *trans*-cinnamaldehyde (TC), eugenol (EUG) and β-resorcylic acid (BR) as a wash treatment for reducing *Salmonella* spp. on tomatoes was investigated. Plum tomatoes inoculated with a six-serotype mixture of *Salmonella* (10^8 CFU) were subjected to washing in sterile deionized water (control) or deionized water containing chlorine (100 ppm), CAR (0.25 and 0.75%), TC (0.5 and 0.75%), EUG (0.25 and 0.75%), or BR (0.75 and 1.0%) for 15 sec, 1 min, and 3 min. The plant molecules were more effective ($P<0.05$) in reducing *Salmonella* on tomatoes compared to washing in water and chlorine. Both concentrations of CAR and TC, and 0.75% EUG decreased *Salmonella* counts on tomatoes by ~6.0 log CFU/ml at 1 min. Both concentrations of BR decreased the pathogen on tomatoes to undetectable levels at 3 min of exposure. Washing of tomatoes in deionized water and chlorine for 3 min reduced *Salmonella* by ca. 2.0 and 4.0 log CFU/ml, respectively. No *Salmonella* was detected in the wash water containing the plant molecules or chlorine, whereas a substantial population of the pathogen survived in the control wash water. Moreover, none of the dipping treatments had any effect on the red color of tomatoes ($P>0.05$). Results indicate that CAR, TC, EUG and BR could effectively be used to kill *Salmonella* on tomatoes, but additional studies on sensory and quality characteristics of tomatoes treated with plant molecules are warranted.

1. Introduction

Fruits and vegetables constitute an important portion of the American diet. During the period from 1980 to 2001, the per capita consumption of fresh fruits and vegetables in the United States increased by 19% and 29%, respectively (CARD, 2004). In the US alone, the proportion of outbreaks linked to fresh produce increased from less than 1% of all reported outbreaks with a known food vehicle in the 1970s to 6% in the 1990s (Sivapalasingam et al., 2004). Among the foodborne pathogens in the US, *Salmonella* constitute one of the most prevalent bacteria, causing an estimated 1.6 million foodborne illnesses with annual cost of ~$14 billion (Scharff, 2010). Although salmonellosis has been largely associated with the consumption of contaminated foods of animal origin, fresh tomatoes have been linked to major outbreaks of the pathogen time after time. Multi-state outbreaks involving *Salmonella* serotypes such as *S.* Baildon, *S.* Braenderup, *S.* Javaina, S. Montevideo, *S.* Newport, *S.* Saintpaul and *S.* Typhimurium associated with consumption of tomatoes have been reported. The largest *Salmonella* outbreak in the U.S., associated with *S.* Saintpaul was linked to consumption of hot peppers and possibly tomatoes (CDC, 2008). Epidemiological investigations have revealed that tomatoes can potentially get contaminated with *Salmonella* from a variety of sources, including irrigation water, wash water, food preparation environment and animals (Hanning et al., 2009).

A wide range of chemical sanitizers and physical treatments have been investigated with varying degrees of success for killing *Salmonella* on tomatoes (Beuchat, 1998; Lang et al., 2004). Although chlorine is one of the common sanitizers used in the industry to decontaminate fresh produce, tomatoes washed with chlorine (40–60 ppm) were involved in outbreaks (Wei et al., 1995). In another study, Zhuang et al. (1995) reported that complete inactivation of *Salmonella* on tomatoes was not achieved with 320 ppm chlorine. Moreover, formation of chlorinated organic compounds, such as trihalomethanes from chlorine has raised safety concerns on their potential impact on humans and the environment, thus triggering the search for alternatives to chlorine (Parish et al., 2003).

The use of natural antimicrobial molecules for inactivating pathogenic microorganisms has received renewed attention due to concerns for toxicity of synthetic chemicals (Salamci et al., 2007). Plants have been a source of many natural molecules that contribute to human health and well-being. The antimicrobial properties of plant essential oils have been demonstrated previously (Burt, 2004), and a variety of active components in these oils has been identified. *Trans*-cinnamaldehyde (TC) is an aldehyde present as a major component of

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bark extract of cinnamon (*Cinnamomum zeylandicum*). Carvacrol (CAR) is an antimicrobial ingredient in oregano oil obtained from *Origanum glandulosum*. Eugenol (EUG) is an active ingredient in the oil obtained from cloves (*Eugenia caryophillis*). β-resorcylic acid (BR; 2, 4 dihydroxy benzoic acid) is widely distributed among the angiosperms, and is a secondary metabolite that plays a key role in the biochemistry and physiology of plants (Friedman et al., 2003). All these molecules are classified by the United States Food and Drug Administration as GRAS (generally regarded as safe) (Adams et al., 2004, 2005; Baskaran et al., 2010; Knowles et al., 2005).

Previous research conducted in our laboratory revealed that TC, EUG, and CAR were effective in inactivating major mastitis pathogens in milk (Ananda Baskaran et al. 2009), and TC inactivated *S.* Enteritidis and *Campylobacter jejuni* in chicken drinking water (Kollanoor Johny et al. 2008). These compounds have also been reported to possess antimicrobial activity against *S.* Typhimurium and *S.* Typhimurium DT104 (Feng et al., 2007, Si et al., 2006). Recently, we reported that TC, EUG, CAR and BR increased the sensitivity of *S.* Typhimurium DT104 to several antibiotics (Kollanoor Johny et al., 2010). The objective of this study was to determine the efficacy of TC, CAR, EUG and BR as a wash treatment for reducing *Salmonella* spp. on tomatoes.

2. Materials and methods

2.1. Salmonella strains

The *Salmonella enterica* isolates used in this study included *S.* Montevideo, *S.* Poona, *S.* Newport, *S.* Baildon, *S.* Braenderup, and *S.* Saintpaul. These natural isolates from tomatoes were kindly provided by Dr. Larry B. Beuchat (Center for Food Safety, University of Georgia, Griffin, GA).

2.2. Preparation of inocula

Each *Salmonella* **s**train was cultured separately in 10 ml of tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) and incubated at 37 °C for 24 h with agitation (100 rpm). After three successive transfers, equal volumes of the cultures were combined and sedimented by centrifugation (3600 *g* for 15 min at 4 °C). The pellet was washed two times and resuspended in phosphate buffered saline (PBS; pH 7.0) and used as the inoculum. The bacterial count in the individual cultures and the 6-strain mixture was confirmed by plating 0.1 ml proportions of appropriate dilutions on tryptic soy agar (TSA, Difco) and xylose lysine desoxycholate agar (XLD; Difco) and incubating the plates at 37 °C for 24 h (Kollanoor Johny et al. 2008).

2.3. Preparation and inoculation of tomatoes

Fresh plum tomatoes were purchased from a local supermarket and refrigerated for not more than 24 h. Tomatoes were equilibrated to room temperature (23 ± 2 °C) before testing and washed with 70% ethanol to sterilize and remove wax residue, if there was any present. Further, the tomatoes were washed three times with sterile deionized water to remove any remaining ethanol residue and were allowed to dry in a laminar flow hood for 1 h at 23 ± 2 °C under UV light before inoculation (Lang et al. 2004).

Inoculation of tomatoes was carried out according to the protocol described by Lang et al. (2004). Briefly, tomatoes were placed stem down on autoclaved aluminum foils inside a laminar flow hood. Within a 3-cm diameter circle on the surface of the blossom end of the tomato, 100 μl of the six-strain inoculum (approximately 8.0 log CFU) of *Salmonella* was applied using a micropipette. Care was taken to prevent the application of the inoculum on the blossom scar. Small and approximately equal volumes of inoculum were applied at 15 to 20 locations to help facilitate drying and prevent the inoculum from running down the side of the tomato. The inoculated tomatoes were held in a laminar flow hood to allow the inoculum to dry for 1 h at 23 ± 2 °C.

2.4. Treatment of tomatoes

Each tomato was placed with the blossom end down in separate 5.5" × 9" sterile sampling bags (Fisher Scientific Co LLC, Hanover Park, IL) containing 200 ml of sterile deionized water added with one of the treatments namely, 100 ppm chlorine (Burnett and Beuchat, 2002) (~ 2% NaOCl; Ricca Chemical Company, Arlington, TX), 0.5% TC (Sigma-Aldrich Corp, St. Louis, MO), 0.75% TC, 0.25% CAR (Sigma-Aldrich), 0.75% CAR, 0.25% EUG (Sigma-Aldrich), 0.75% EUG, 0.75% BR (Sigma-Aldrich) or 1.0% BR. All plant molecules except BR were added directly to water to obtain the desired concentrations. BR powder was diluted in dimethyl sulfoxide (DMSO) (Sigma-Aldrich) for better solubility, and added to achieve the desired concentrations. The final concentration of DMSO in dipping solutions was not more than 1%, which did not exert any antimicrobial effect (Ahameethunisa and Hopper, 2010; Nair et al. 2005; Nakamura and Hatanaka, 2002). The concentrations of CAR, TC, EUG and BR were selected based on preliminary experiments conducted in our laboratory. Treatment bags containing only sterile deionized water without any antimicrobial served as controls. In addition, inoculated, but unwashed tomatoes were also included to determine the efficiency of inoculation and obtain baseline *Salmonella* counts (baseline samples).

Each bag containing one tomato submerged in the control or treatment solution (25 °C) was placed in a reciprocal water bath shaker (Model R76; New Brunswick Scientific, Edison, NJ) and shaken for 15 s, 1 min or 3 min. At the end of each specified time, the tomato was aseptically transferred to a second bag containing 50 ml of Dey-Engley neutralizing broth (DE neutralizing broth; Difco, Becton Dickinson, Sparks, MD) (Singh et al. 2002). Each tomato was then hand rubbed for 1 min in DE broth before determining the surviving *Salmonella* populations (Lang et al. 2004). Triplicate samples of each treatment, control and baseline were included in each experiment and the entire experiment was replicated two times.

2.5. Microbiological analyses

One ml of DE neutralizing broth from each bag was serially diluted (1:10) with 9 ml of sterile PBS, and 0.1-ml portions from appropriate dilutions were spread plated on duplicate TSA and XLD plates. A volume of 0.1-ml of wash suspension from each sampling bag was also directly plated on duplicate TSA and XLD plates without serial dilutions. The plates were incubated at 37 °C for 24 h before counting the colonies. In addition, a volume of 1 ml of wash solution from each bag was transferred to separate 250-ml Erlenmeyer flasks containing 100 ml of sterile TSB, and incubated at 37 °C for 24 h. Following enrichment in TSB, the culture was streaked on XLD plates, and incubated at 37 °C for 24 h. Representative colonies of bacteria from TSA and XLD plates were confirmed for *Salmonella* using the *Salmonella* rapid detection kit (Microgen Bioproducts Ltd, Camberley, UK).

2.6. Tomato color analysis

The red color of tomatoes can be measured by colorimetry and quantitatively reported as a^* values. High positive a^* values indicate more red color, while lower a^* values denote decreased red color (Batu, 2004). Four tomatoes per dipping treatment (water control, undipped control, chlorine 100 ppm, 0.25% CAR, 0.75% CAR, 0.5% TC, 0.75%TC, 0.25% EUG, 0.75% EUG, 0.75% BR and 1.0% BR; $N = 44$) were included. Tomatoes were treated for a minute in respective treatments, and two separate a^* values were taken from each side of the tomato on day 0 and 3 of storage at 4 °C using a MiniScan® EZ portable reflected-color measurement spectrophotometer (HunterLabs, Reston, VA). The values were pooled from each tomato, and averaged ($n = 4$) to obtain the mean a^* value of tomatoes subjected to each treatment.