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Enhanced inactivation of *Salmonella* Typhimurium from blueberries by combinations of sodium dodecyl sulfate with organic acids or hydrogen peroxide

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ABSTRACT

Increasing consumption of raw blueberries has led to the need for improved food safety in the berry fruit industry. This study was undertaken to evaluate an enhanced *Salmonella* inactivation on blueberries by washing with sodium dodecyl sulfate (SDS) in combination with one of common antimicrobial agents (chlorine, lactic acid, acetic acid, citric acid and hydrogen peroxide). In addition, the impacts of most effective treatments on apparent quality, total phenolic, anthocyanin content, mold and yeast, and *Salmonella* survivors during the storage of blueberries were also determined. Maximum reductions of *Salmonella* after washing with 0.5 mg/ml acetic acid plus 5000 ppm SDS and 200 ppm hydrogen peroxide containing 5000 ppm SDS were 4.0 and 4.2 $\log_{10}$ CFU/g, respectively. Addition of SDS enhanced *Salmonella* inactivation of the acetic acid or hydrogen peroxide solutions. These treatments showed similar *Salmonella* reductions with 200 ppm chlorine ($P > 0.05$). None of these washings decreased the total phenolic, anthocyanin content and apparent quality. Following the two effective antimicrobial washes, 0.5 mg/ml acetic acid plus 5000 ppm SDS and 200 ppm hydrogen peroxide containing 5000 ppm SDS, populations of *Salmonella* on blueberries remained constant throughout the 3-day storage with no appreciable growth. Both treatments decreased counts of yeasts and molds throughout the storage. Therefore, the use of 0.5 mg/ml acetic acid plus 5000 ppm SDS, 200 ppm hydrogen peroxide in combination with 5000 ppm SDS may have practical potential as an alternative to the use of chlorine-based washing solution for blueberries.

1. Introduction

Salmonella is one of the leading causative microorganisms of foodborne illness in the United States and many other countries. Scallan, Hoekstra, Angulo, Griffin, and Tauxe (2011) estimated that 31 pathogens caused 228,744 hospitalizations annually, 35% of which resulted from *Salmonella*. Outbreaks of salmonellosis have been linked to a wide variety of fresh fruits and vegetables including apple, cantaloupe, alfalfa sprout, mango, lettuce, cilantro, unpasteurized orange juice, tomato, melon, celery and parsley (Pui et al., 2011). A total of 127 persons were infected with *Salmonella* Braenderup on the mangoes from Agricola Daniella. 33 ill persons were hospitalized in 2012 (CDC, 2012). *Salmonella* Typhimurium and *Salmonella* Newport contaminated cantaloupes originating from Chamberlain Farms caused 94 individuals to become ill, resulting in 3 deaths (CDC, 2012).

Since blueberries are generally consumed raw or minimally processed in order to increase the shelf life of the product, there are many opportunities for contamination of the fruits during production, such as irrigation water, soil, equipment, pickers, and food handlers. In 1984, an outbreak of listeriosis was associated with fresh blueberries in Connecticut (Ryser, 1999). A more recent outbreak of hepatitis A in New Zealand was linked to the consumption of raw blueberries, which were likely contaminated from infected food handlers or fecally contaminated groundwater (Calder et al., 2003). According to the Foodborne Outbreak Online Database (2013), a confirmed outbreak of *Salmonella* Muenchen on blueberries caused 14 people to become ill in June 2009, which implicates that *Salmonella* may also become important contamination source for blueberries.

In the berry industries, postharvest blueberries are usually washed or sprayed with chlorinated water containing 50–200 ppm active chlorine to reduce microorganisms (Good Agricultural and Manufacturing (Handling) Safety and Food Defense Practices, 2010). However, this chlorine-based washing solution has shown limited efficacy to deactivate foodborne pathogens. Five minutes of exposure to 100 ppm chlorine was reported to reduce populations of bacteria, yeast, and mold on blueberries by only 0.83, 0.77, and 0.61 $\log_{10}$ CFU/g, respectively (Crowe, Bushway, & Bushway, 2005). The chlorine-based washing solutions also bring safety risks such as the undesirable chlorine by-products of chloroform ($CHCl_3$), suspected for carcinogenic or mutagenic effects (Artes, Gomez, Aguayo, Escalona, & Artes-Hernundez, 2009; Nieuwenhuijsen, Toledano, & Elliot, 2000). Scientists have tried to find alternative methods such as citric acid or hydrogen peroxide to protect blueberries from decaying, to prolong shelf life, and to secure

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product safety (Crowe, Bushway, Bushway, Davis-Dentici, & Hazen, 2007; Crowe, Bushway, & Bushway, 2005; Sapers & Simmons, 1998; Sapers & Sites, 2003; Shapiro & Holder, 1960). Although treatments containing higher concentrations of citric acid and hydrogen peroxide may have lethal or inhibitory effects on microorganisms, the use of these treatments in blueberry processing should be limited due to adverse changes in flavor and surface color (Abee & Wouters, 1999; Beuchat, 1998; Wesche, Gurtler, Marks, & Ryser, 2009).

Sodium dodecyl sulfate (SDS) is a surface-active compound found in the washing detergent. This compound makes the liquid spread more easily and lowers the interfacial tension between two liquids or between a liquid and a solid. Previous research suggested the additions of SDS to levulinic-acid enhanced removal of *Salmonella* on chicken breast meats (Zhao, Zhao, & Doyle, 2009), and combinations of SDS with chlorinated water also improved inactivation of human norovirus surrogates on produce (Predmore & Li, 2011). So the objective of this study was to develop an antimicrobial washing solution using a common sanitizer (chlorine, lactic acid, acetic acid, citric acid and hydrogen peroxide) and SDS to enhance the *Salmonella* inactivation on blueberries. In addition, the impacts of most effective treatments on sensory quality, total phenolic, anthocyanin content, molds and yeasts, and *Salmonella* survivors during the storage of blueberries were also determined.

2. Materials and methods

2.1. Bacterial strains and inoculum preparation

The culture of *Salmonella* enterica serovar Typhimurium DT 104 used for this study was obtained from the culture collection in the Department of Animal and Food Sciences at the University of Delaware (Newark, DE). Stock cultures on Tryptic soy agar (TSA; Difco Laboratories, Becton Dickinson, Spark, Md) were stored at 4 °C. Single colony of stock culture from TSA plate was cultured into tryptic soy broth (TSB, Difco Laboratories, Becton Dickinson, Spark, MD) and grown at 37 °C for 24 h before inoculation on the blueberries. Populations of the cell culture were determined by serially diluting suspensions in sterile 0.1% peptone water (Difco Laboratories) and spread plating 0.1 ml on the xylose lysine deoxycholate (XLD; Difco Laboratories) plate. Culture of *S.* Typhimurium yielded an approximate population of 10^8 CFU/ml (Bialka & Demirci, 2007).

2.2. Contamination of blueberries' surface and washing procedures

Fresh blueberries were obtained from a local market on the day of experimentation. Intact surfaces were selected for inoculation. Inoculation was achieved by applying 25 μl of inoculum (6 to 10 small droplets) onto the surface of each blueberry. After inoculation, blueberries were left in a laminar flow hood at room temperature (21 ± 1 °C) for about 2 h to allow for attachment of the microorganisms. Inoculated blueberries had approximately 10^6 CFU/g of *Salmonella*.

The washing solutions included: chlorine (4, 12.5, 25, 50 and 100 ppm, prepared from sodium hypochlorite, Sigma-Aldrich, Inc., St. Louis, MO, USA), organic acids (lactic acid, acetic acid, and citric acid at 0.05 mg/ml or 0.5 mg/ml, Sigma-Aldrich, Inc., St. Louis, MO, USA) and hydrogen peroxide (50, 100 and 200 ppm, prepared from 30% hydrogen peroxide, Sigma-Aldrich, Inc., St. Louis, MO, USA), as well as these antimicrobial solutions combined with SDS (50, 500, 5000 ppm, Sigma-Aldrich, Inc., St. Louis, MO, USA) were also tested. Distilled water (DI) washing served as negative control. Blueberries at 4 g were submerged in 80 ml of the rinse solution with continuous agitation provided by starring bar in a beaker for 1 min or 5 min. The 5 min washing was used in initial tests to determine the best treatments, based on previous research of Crowe et al. (2005). After identifying the most effective treatments, 80 g of blueberries was used for further evaluation on the antimicrobial efficacy of these effective treatments and their impacts on blueberries' qualities, as described in Sections 2.4, 2.5, and 2.6. In addition, 1 min washing time was studied for the best effective treatments to determine if a shorter time was feasible to obtain effective *Salmonella* inactivation.

2.3. Enumeration of Salmonella

After treatments, blueberries were placed in a sterile stomacher sample bag containing 10 ml of elution buffer (phosphate-buffered saline [PBS]) and pummeled in a stomacher (Colworth Stomacher 400, A.J. Seward and Co., Ltd., London, UK) for 2 min at medium speed. For the quantification of surviving bacteria, 1 ml aliquots from 10 ml of homogenate were plated on three XLD agar plates. Plates were incubated at 37 °C for 24 h before presumptive-positive colonies were counted (Andrews, Jacobson, & Hammack, 2011). The microbial population detection limit was 0.39 $\log_{10}$ CFU/g.

A modified *Salmonella* enrichment experiment was carried out by the method previously used in the lab (Lu & Wu, 2010). A combination of 1 ml of the blueberry homogenates in PBS and 9 ml of preenrichment broth (TSB) was incubated at 37 °C for 24 h. After incubation, 1 ml of TSB was plated on three XLD agar plates. Plates were incubated at 37 °C for 24 h and examined for the presence of *Salmonella* colonies.

2.4. Effect of washing treatments on the physical properties of blueberries

Fresh blueberry color was determined by a color reader (Minolta model CR-10, Minolta Camera Co., Ltd., Osaka, Japan) to determine the following color values: L* (brightness/darkness), a* (redness/greenness), and b* (yellowness/blueness). Measurements were taken at three different parts of treated and untreated un-inoculated blueberries. Texture was measured by a TA.XT2 texture analyzer (Texture Technology Corp., Scarsdale, N.Y., U.S.A.) using a TA-91 Kramer shear probe with a rounded end. For the pH measurement, 5 g blueberries were placed in sterile stomacher sample bags and homogenized in a stomacher for 2 min at medium speed. The pH values of homogenates were measured by a pH meter (FiveEasy FE20, Mettler-Toledo AG, Greifensee, Switzerland).

2.5. Total phenolic and anthocyanin content

The total phenolic contents of untreated and treated blueberries were determined as described by Tsai, Chien, Lee, and Tsai (2008). The gallic acid was used as a standard. Twenty μl of supernatants from centrifuged homogenates of treated and untreated blueberries, 180 μl of distilled water, 100 μl of the Folin–Ciocalteau reagent (Sigma-Aldrich, Inc., St. Louis, MO, USA), and 0.5 ml of a 20% sodium carbonate (Sigma-Aldrich, Inc., St. Louis, MO, USA) solution composed the reaction mixtures. The reaction mixtures were covered, vortexed vigorously, and allowed to react at room temperature for 2 h in microcentrifuge tubes. 200 μl of these mixtures was added into each blank well of a clear 96-well plate. The absorbance was measured at 765 nm by the Synergy 2 multimode microplate reader (BioTek Instruments, Inc., Winooski, VT) and the total phenolic content was calculated as mg gallic acid (Sigma-Aldrich, Inc., St. Louis, MO, USA) equivalent/100 g berries.

The method for determination of total anthocynin content was performed according to the procedure described by Qi et al. (2011) with modifications. Fresh blueberry samples at 5 g and 30 ml of 80:20 (v/v) methanol–water solution containing 0.1 ml/l acetic acid were added to a tube and homogenized by ultra-turrax for 2 min. The mixture (total volume of 30 ml) was placed in the dark for 1 h and then sonicated for 15 min. After centrifugation at 5000 rpm for 30 min at room temperature, the volume of the supernatants was recorded. The total anthocyanin content was measured by the pH differential method with some modification. The anthocyanin extract was dissolved in a 0.025 M potassium chloride buffer (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5) with a dilution factor at 6.0. The absorbance of each