Food Control 84 (2018) 106–110

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

A decontamination approach using a combination of bisulfate of soda and peracetic acid against *Listeria innocua* inoculated on whole apples

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ARTICLE INFO

Article history:
Received 7 June 2017
Received in revised form
25 July 2017
Accepted 26 July 2017
Available online 26 July 2017

Keywords:
Apple
Listeria innocua
Antimicrobial
Bisulfate of soda
Peracetic acid

Chemical compounds studied in this article:
Bisulfate of soda (PubChem CID: 516919)
Peracetic acid (PubChem CID: 6585)

ABSTRACT

Developing novel and effective antimicrobial methods is imperative for ensuring food safety during commercial apple production. In the present study, the bactericidal effects of dipping apples into a combination of bisulfate of soda (BS) and peracetic acid (PAA) was investigated using *Listeria innocua* as a surrogate inoculated to whole apples. Decontamination treatments included washing with water as control, chlorine at 150 ppm, 1% BS with 60 ppm PAA, 3% BS with 60 ppm PAA, and 3% BS with 60 ppm PAA and a surfactant sticker (0.1% Tween). After dipping with antimicrobial solutions for 2 min, apples were stored over a time period of up to 2 weeks and *L. innocua* survivors were enumerated from apple core collected from 30 min, 1 day, 7 days, and 14 days. Washing with water showed little antimicrobial effect; only a 1.52 $\log_{10}$-cycle reduction was observed after 14 days (*L. innocua* population at 0 and 14 days: 5.96 and 4.44 log CFU/g, respectively), indicating that washing was not sufficient to control *L. innocua* on apple. When apples were treated with chlorine, *L. innocua* populations were reduced to 3.58, 2.19 log CFU/g after 1 and 7 days, respectively, but afterward increased to 3.80 log CFU/g at 14 days of storage. In contrast, the combined treatment of BS and PAA resulted in marked bactericidal activities ($\log_{10}$-cycle reduction by 1% BS with 60 ppm PAA after 30 min, 1, 7, and 14 days: 2.57, 2.70, 5.45, and 4.30 log CFU/g, respectively; $\log_{10}$-cycle reduction by 3% BS with 60 ppm PAA: 3.66, 5.24, 5.50, and 5.56 log CFU/g). Adding a surfactant sticker did not result in a significant increase in antimicrobial effects thus 3% BS with 60 ppm PAA would be an optimal treatment for use in the apple industry. This combined decontamination method has important advantages including consumer and industry preference for natural compound, its potential application to industry, and cost-effectiveness. The combined treatment of BS and PAA may be a useful decontamination method for improving the microbiological safety in whole apples.

1. Introduction

Fresh fruits are an important part of healthy diet but have occasionally been implicated in foodborne disease outbreaks by pathogenic bacteria which can cause clinical disease in humans. Since fresh fruits are usually consumed as raw without further processing to reduce pathogen contamination, they pose a greater food safety risk than foods receiving lethality processing or other antimicrobial treatment (Abadias, Usall, Anguera, Solsona, & Viñas, 2008; Callejón et al., 2015). Food safety of fresh fruits is of considerable concern and ensuring the microbiological safety of fresh fruit is of primary interest to the food industry. Apples are one of the more popular fresh fruits and are widely consumed throughout the world but they could be a vector for foodborne diseases, such as human listeriosis (Centers for Disease Control and Prevention, 2015). For example, whole caramel apples were the cause of a listeriosis outbreak that infected 35 people (34 were hospitalized and listeriosis contributed to at least 3 of the 7 deaths) from 12 states in 2014–2015 (Centers for Disease Control and Prevention, 2015).

Traditionally, chlorine treatments were the most frequently

Abbreviations: BS, bisulfate of soda; PAA, peracetic acid.

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http://dx.doi.org/10.1016/j.foodcont.2017.07.036
0956-7135/

used measure to reduce pathogen contamination in the fresh produce industry but there is a trend towards removing chlorine as a treatment due to its environmental and health risk (Ölmez & Kretzschmar, 2009a). One of the alternative measures to chlorine is using organic acids such as citric acid, lactic acid, or peracetic acid (PAA) (Ölmez & Kretzschmar, 2009b). Peracetic acid, a mixture of acetic acid and hydrogen peroxide, is a sanitizer possessing antimicrobial effects against a wide spectrum of bacteria, virus, and fungi, and has been used to control bacteria in foods as well as on equipment/utensils in the food and poultry industries (King et al., 2005; Kitis, 2004; Ölmez & Kretzschmar, 2009a; Warburton, 2014).

In addition to being an antimicrobial, organic acids can also potentiate the effect of sanitizers mainly by decreasing overall pH (Ricke, 2003). Bisulfate of soda (BS), also referred to as a sodium hydrogen sulfate, sodium acid sulfate, and sodium bisulfate, is a dry acid product that has potential value as a means to lower the risk of pathogen contamination in the food industry (Fan, Sokorai, Liao, Cooke, & Zhang, 2009; Kassem, Sanad, Stonerock, & Rajashekara, 2012; Rubinelli, Kim, Park, Roto, & Ricke, 2017; Yang, Li, & Slavik, 1998). Bisulfate of soda dissociates into sulfate ions, sodium, and hydrogen (pKa: 1.99 and it starts to buffer around pH 2), and possesses the distinctive advantage of lowering the pH without exhibiting detectable sour flavor, thus representing a better choice for such applications compared to other acids (Sun et al., 2008). Bisulfate of soda is a natural food acid and was categorized as GRAS (Generally Recognized As Safe, 21 CFR 582.1095) by the Food and Drug Administration (FDA) and therefore is considered suitable for use in human foods (Li, Slavik, Walker, & Xiong, 1997). It has been widely used as an acidifier and anionic ingredient throughout the food and animal industries including sauces and dressings, soups, prepared meals, vegetables and fruits, beverages, pet food, poultry feed, drinking water, dairy and swine manure, and livestock bedding (Calvo, Gerry, McGarvey, Armitage, & Mitloehner, 2010; Kassem et al., 2012; Sun et al., 2008).

Further development of antimicrobial applications is expected to focus on treatment combinations of two or more antimicrobials where combined effects are predicted to achieve synergism by exhibiting greater bactericidal effects than individual antimicrobials and this is so-called hurdle technology (Chen & Jiang, 2014; Davidson & Branen, 2005; Ricke, Kundinger, Miller, & Keeton, 2005). Peracetic acid has clearly been the preferred sanitizer to use with an organic acid in the past since PAA is an equilibrium reaction with acetic acid and hydrogen peroxide. To the best of our knowledge, no study has addressed the practical antimicrobial effects of the combined treatment of BS with other acidulants on whole apple. The object of the present study was to determine the efficacy of antimicrobial dips using BS and PAA to reduce *Listeria* contamination by using *Listeria innocua* as a non-pathogenic surrogate for pathogenic *Listeria monocytogenes* (Milillo et al., 2012; O'Bryan, Crandall, Martin, Griffis, & Johnson, 2006) when inoculating whole apples for simulating industrial conditions to assess potential effectiveness for decreasing microbial contamination.

2. Materials and methods

2.1. Bacterial strains used in this study

Listeria innocua ATCC 33090 served as a non-pathogenic surrogate for *L. monocytogenes* and was grown with trypticase soy broth (TSB; Becton Dickinson and Company, Sparks, MD) with 1% glucose at 37 °C for 24 h. After incubation, the *L. innocua* pure culture was washed twice with sterile phosphate buffered saline using centrifugation at 15,557 RCF for 5 min (Centrifuge 5804 R, Eppendorf, Germany). After centrifugation, the final cell pellet was resuspended in the same buffer and utilized as an inoculum.

2.2. Inoculation of Listeria innocua

Whole Granny Smith apples were supplied without wax treatments. Apples were fully matured and if the apples had a rotten spot, they were removed. Prior to performing the experiment, the absence of *L. innocua* was confirmed with enrichment in TSB for overnight followed by streaking onto Palcam agar (Merck, Darmstadt, Germany). Samples without any presumptive colonies on Palcam agar were subjected to the experiment. One hundred apples were utilized for each experiment. Prior to conducting the experiment, all apples were sanitized with 100 ppm chlorine to eliminate any surface bacteria that might interfere with the test results. Apples were dipped in an inoculum solution. After inoculation, apples were allowed to sit and dry individually on trays with the calyx and stem horizontally so that there would be no contact among individual apples. The apples were dried for 24 h at 45 °F (7.2 °C) to attach bacteria.

2.3. Antimicrobial treatment

Following inoculation, each set of 20 apples was subjected to one of five treatments: 1) distilled water as control, 2) chlorine at 150 ppm at a pH of 6.5 with citric acid, 3) 1% BS (Jones-Hamilton Co, Walbridge, Ohio) with 60 ppm PAA (TSUNAMI 100® Ecolab Inc. St. Paul, Minnesota), 4) 3% BS with 60 ppm PAA, and 5) 3% BS with 60 ppm PAA with a surfactant (0.1% Tween™ 20 Croda Inc., Edison, New Jersey). Each set of 5 whole apples was treated by being fully submerged in water or antimicrobial solutions for 2 min at room temperature. A fresh solution was supplied for each group to eliminate carryover of surrogate organisms. After treatment, apples were stored at 7.2 °C while waiting to be tested in typical 100 cc/100 in^2/24 h oxygen transmission rate bags widely used in the fresh cut industry. Five apples from each group were collected at 30 min, 1 day, 7 days, and 14 days and *L. innocula* populations were enumerated as described below (section 2.4).

2.4. Enumeration of survivors

After the designated storage time at 7.2 °C, apple cores were aseptically removed. All coring equipment and devices were sanitized between each coring using an iodine dip followed by immersion in alcohol and burning off the alcohol. The core was pulverized and homogenized using a stomacher (Bagmixer 400 Model P, Interscience Laboratories Inc., Cummings Park, Woburn, MA) at 260 ppm for 2 min with 10 times the volume of the Dey-Engley neutralizing buffer (Difco Laboratories, Detroit, MI) to terminate the bactericidal activity. One ml of each homogenized sample was serially diluted in 9 ml of sterile PBS, and 100 μl of diluents were spread-plated onto trypticase soy agar (Becton Dickinson and Company, Sparks, MD). To recover injured bacteria, each spread-plated agar plate was incubated at room temperature for 4 h before overlaying the plate with a Palcam agar (Kang & Fung, 2000). The plates were incubated for up to 3 days at 35 °C and typical *L. innocua* colonies were enumerated.

2.5. Statistical analysis

Analysis of variance (ANOVA) was performed using the general linear models procedure in the SAS statistical analysis package (Version 9.13; SAS Institute Inc., Cary, NC, USA). When ANOVA indicated a significant result ($P < 0.05$), differences between the mean values were determined using Tukey's multiple range test.