Food Control 82 (2017) 126–135

Contents lists available at ScienceDirect

Food Control

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

Antimicrobial efficacy of vacuum impregnation washing with malic acid applied to whole paprika, carrots, king oyster mushrooms and muskmelons

Jun-Won Kang [a, b], Dong-Hyun Kang [a, b, *]

[a] Department of Food and Animal Biotechnology, Department of Agricultural Biotechnology, Center for Food and Bioconvergence, and Research Institute for Agricultural and Life Sciences, Seoul National University, Seoul, 08826, Republic of Korea
[b] Institutes of Green Bio Science & Technology, Seoul National University, Pyeongchang-gun, Gangwon-do, 25354, Republic of Korea

ARTICLE INFO

Article history:
Received 5 January 2017
Received in revised form
24 May 2017
Accepted 25 May 2017
Available online 26 May 2017

Keywords:
Fresh produce
Salmonella Typhimurium
Escherichia coli O157:H7
Listeria monocytogenes
Sanitizer
Organic acid
Vacuum impregnation
Surface roughness

ABSTRACT

Antimicrobial effect of vacuum impregnation (VI) applied to organic acid washing against *Salmonella* Typhimurium, *Escherichia coli* O157:H7 and *Listeria monocytogenes* on paprika fruit, carrots, king oyster mushrooms and muskmelons was investigated. Samples were treated with intermittent VI with 21.3 kPa and compared with dipping washing in 2% malic acid. The initial sample pathogen levels were approximately 10^5–10^7 CFU/cm^2. Enumerations of the three pathogens on paprika and carrots treated with VI washing were reduced to below the detection limit (= 1 $\log_{10}$ CFU/cm^2) after 3–5 min and 15–20 min, respectively. For each time point where populations of the three pathogens were reduced to below the detection limit by VI treatment, populations of 1.2–1.9 log CFU/cm^2 and 2.5 to 2.8 log CFU/cm^2 survived on paprika and carrots, respectively, when subjected to dipping treatment. After 20 min of dipping treatment, surviving populations of the three pathogens ranged from 3.5 to 4.1 and 3.3 to 4.4 log CFU/cm^2 on king oyster mushrooms and muskmelons, respectively. After 20 min of VI treatment, surviving populations of the three pathogens ranged from 3.0 to 3.6 log and 3.1 to 4.1 log CFU/cm^2, respectively, on king oyster mushrooms and muskmelons. Additionally, there were no significant ($P \geq 0.05$) differences in pathogen reductions between dipping and VI treatment for both king oyster mushrooms and muskmelons. King oyster mushrooms (R_a = 6.02 ± 1.65 μm) and muskmelons (R_a = 11.43 ± 1.68 μm) had relatively large roughness values compared to those of paprika (R_a = 0.60 ± 0.10 μm) and carrots (R_a = 2.51 ± 0.50 μm). Scanning electron photomicrographs showed many deep protected sites in king oyster mushrooms and muskmelons with many microbes located deep in these sites following VI treatment. Instrumental color, texture and titratable acidity values of paprika and carrots subjected to VI washing treatment with 2% malic acid for 5 and 20 min were not significantly ($P \geq 0.05$) different from those of untreated control samples during 7 day storage.

1. Introduction

While fresh produce is popular worldwide and its consumption has increased over the past two decades, a number of foodborne illness outbreaks associated with the consumption of fresh produce have also occurred every year (CSPI, 2009; Callejón et al., 2015; Warriner, Huber, Namvar, Fan, & Dunfield, 2009). Since most fresh produce is minimally processed or eaten raw and does not go through a kill step treatment such as cooking, pathogen contamination can pose a serious risk (Goodburn & Wallace, 2013). Pathogen contamination can occur during any of many steps along the farm-to-consumer continuum such as untreated manure used for fertilization, contaminated irrigation water, infected workers, the presence of domestic or wild animals and birds, and contaminated containers and tools used in harvesting, packing, transporting, or processing (FDA, 2014; Jiang, Chen, & Dharmasena, 2014). Although there are preharvest strategies which may help decrease the risk of contamination such as GAPs during growing and harvesting, there is still much reliance on produce decontamination strategies applied by the processing industry (Goodburn & Wallace, 2013).

* Corresponding author. Department of Agricultural Biotechnology, Seoul National University, Seoul, 08826, Republic of Korea.
E-mail address: kang7820@snu.ac.kr (D.-H. Kang).

http://dx.doi.org/10.1016/j.foodcont.2017.05.039
0956-7135/

Generally, the food industry applies a washing process such as submersion or spray with chlorinated water containing 50–200 ppm to fresh produce to control pathogens (Wu & Kim, 2007). Although many past studies have demonstrated that this washing process cannot eliminate pathogens on fresh produce, it is still critically important in fresh produce processing as it provides a crucial chance to focus on pathogen inactivation and remove soil, dust and insects from fresh produce in the absence of practical strategies which could help to reduce the risk of fresh produce without causing significant deterioration of produce quality (Gil, Selma, López-Gálvez, & Allende, 2009; Huang, Ye, & Chen, 2012; Luo et al., 2012; Niemira, 2008, 2012). The efficacy of washing is mainly influenced by surface properties of produce (Fransisca & Feng, 2012; Wang, Feng, Liang, LUO, & Malyarchuk, 2009). In part, the inefficacy of aqueous sanitizers is thought to be due to lack of ability to access protected sites (such as cut surfaces, stomata, bacterial aggregates and crevices) on the surface of fresh produce (Burnett & Beuchat, 2001; Olaimat & Holley, 2012). Because of this, it is crucial to develop effective sanitization strategies to control pathogens on produce surfaces and thus reduce foodborne illness outbreaks related to consumption of fresh produce.

To enhance washing efficacy, we applied vacuum impregnation (VI) to the washing process as described in our previous study (Kang & Kang, 2016). VI is a useful technique to quickly exchange the internal gas or liquid of a porous product occluded in open pores with an external liquid phase by the action of a hydrodynamic mechanism (HDM) promoted by pressure changes (Fito, Andres, Chiralt, & Pardo, 1994). We postulated that VI could deliver sanitizers to protected sites and thus washing efficacy would be enhanced. Our previous study (Kang & Kang, 2016) demonstrated that washing efficacy could be enhanced when VI was incorporated with malic acid washing against *Salmonella* Typhimurium and *Listeria monocytogenes* on broccoli. In VI treatment, the decompression level was proportionate to washing efficacy and intermittent treatment was an important factor for effective application of VI to the washing process to help ensure food safety.

The objective of the present study was to extend application of the VI washing technique to include various other types of fresh produce and thus compare its efficacy on these subjects. In common with our previous study (Kang & Kang, 2016), malic acid was chosen as a representative organic acid to use as an alternative sanitizer in place of chlorinated water which has drawbacks such as rapid depletion under conditions of high organic loading and formation of carcinogenic halogenated by-products generated by reaction with organic matter (Wang, Feng, & Luo, 2006). Also, *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* were used as target pathogens because they are of great concern in minimally processed fresh produce (Sagong et al., 2011).

2. Materials & methods

2.1. Bacterial cultures and cell suspension

Three strains each of *S.* Typhimurium (ATCC 19585, ATCC 43971, and DT 104), *E. coli* (ATCC 35150, ATCC 43889 and ATCC 43890) and *L. monocytogenes* (ATCC 19111, ATCC 19115, and ATCC 15313) were provided by the bacterial culture collection of the School of Food Science, Seoul National University (Seoul, South Korea), for this study. Stock cultures were prepared by growing strains in 5 ml of tryptic soy broth (TSB; Difco, BD) at 37 °C for 24 h, combining 0.7 ml with 0.3 ml of sterile 50% glycerol and then storing at −80 °C. Working cultures were streaked onto Tryptic Soy Agar (TSA; Difco, BD), incubated at 37 °C for 24 h and stored at 4 °C for less than 1 mo. Each strain of *S.* Typhimurium, *E. coli* and *L. monocytogenes* was cultured in 10 ml TSB at 37 °C for 24 h, harvested by centrifugation at 4000*g* for 20 min at 4 °C and washed three times with sterile 0.2% peptone water (PW, Bacto, Sparks, MD). The final pellets were resuspended in 10 ml 0.2% PW, corresponding to approximately 10^7–10^8 CFU/ml. Suspended pellets of all strains of the three pathogens were combined into a mixed culture cocktail for use in this study.

2.2. Sample preparation and inoculation

Whole fresh paprika (*Capsicum annuum* L.), carrots (*Daucus carota* subsp. *sativus*), king oyster mushrooms (*Pleurotus eryngii*) and muskmelons (*Cucumis melo* L.) used in this evaluation were purchased from a local market (Seoul, South Korea) and stored at refrigerator temperature (4 ± 2 °C) until experiments were conducted. Intact portions of produce surfaces were cut into 2 by 5 cm (=10 cm^2) pieces. Samples were placed on sterile aluminum foil in a laminar flow biosafety hood and 0.1 ml of previously described culture cocktail was evenly inoculated onto the surface of samples by depositing small droplets at 15–20 locations with a micropipettor (Chen & Zhu, 2011). The inoculated samples were dried for 2 h in the laminar flow biosafety hood at room temperature (22 ± 2 °C) to allow attachment of bacteria, and used in each experimental trial.

2.3. Procedure of treatment

For simple dipping treatment, inoculated samples were immersed in 1 L glass beakers containing 300 ml of 2% malic acid (99.0%; Samchun Chemical Co. Ltd., Pyeongtaek, Korea, pH 2.16) for 3, 5, 10, or 20 min at room temperature (22 ± 2 °C).

For VI treatment, inoculated samples were immersed in 1 L glass beakers containing 300 ml of 2% malic acid and immediately treated with VI in a vacuum oven (OV-11, JEIO TECH Co., Ltd., Daejeon, Korea) for 3, 5, 10, 15, or 20 min at room temperature (22 ± 2 °C). In this treatment, intermittent VI of 21.3 kPa (=3.1 psi) was applied because we found it is more effective than continuous vacuum treatment based on our previous study (Kang & Kang, 2016). Intermittent treatment was comprised of a collection of 5 min treatment cycles, each of which consisted of 2.5 min vacuum treatment followed by 2.5 min atmospheric pressure (=101.3 kPa or 14.7 psi) treatment, except for 3 min treatment (which consisted of 1.5 min vacuum treatment followed by 1.5 min atmospheric pressure treatment). Therefore, 5, 10, and 20 min of intermittent treatment had 1, 2, and 4 cycles, respectively. Only one sample was subjected to each treatment and all experiments were performed using a reticulated stainless steel instrument to keep the samples submerged to prevent their rising to the top of the washing solution.

2.4. Bacterial enumeration

For enumeration of pathogens, treated samples were immediately transferred into sterile stomacher bags (Labplas Inc., Sainte-Julie, Quebec, Canada) containing 100 ml of Dey-Engley (DE) neutralizing broth (Difco) after treatment. Stomacher bags containing treated samples were homogenized with a stomacher (EASY MIX, AES Chemunex, Rennes, France) for 2 min. After homogenization, 1 ml aliquots of stomached samples were tenfold serially diluted in 9 ml of sterile 0.2% buffered peptone water and 0.1 ml aliquots of samples or diluents were spread-plated onto selective media. Xylose lysine desoxycholate agar (XLD; Difco), Sorbitol MacConkey Agar (SMAC; Difco) and Oxford agar base with Bacto Oxford antimicrobial supplement (MOX; Difco) were used as