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Enhanced antimicrobial effect of organic acid washing against foodborne pathogens on broccoli by vacuum impregnation

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ABSTRACT

This study was undertaken to evaluate the effect of vacuum impregnation applied to the washing process for removal of *Salmonella* Typhimurium and *Listeria monocytogenes* from broccoli surfaces. Broccoli was inoculated with the two foodborne pathogens and treated with simple dipping washing or with vacuum impregnation in 2% malic acid for 5, 10, 20, or 30 min. There were two methods of vacuum impregnation: continuous and intermittent. After 30 min of 101.3 kPa (=14.7 psi, simple dipping), 61.3 kPa (=8.9 psi), and 21.3 kPa (=3.1 psi) of continuous vacuum impregnation treatment, there were 1.6, 2.0, and 2.4 $\log_{10}$ CFU/g reductions of *S.* Typhimurium and 1.5, 1.7, and 2.3 $\log_{10}$ CFU/g reductions of *L. monocytogenes*, respectively. After 30 min of 101.3, 61.3, and 21.3 kPa of intermittent vacuum impregnation treatment, there were 1.5, 2.3, and 3.7 $\log_{10}$ CFU/g reductions of *S.* Typhimurium and 1.6, 2.1, and 3.2 $\log_{10}$ CFU/g reductions of *L. monocytogenes*, respectively. Scanning electron photomicrographs showed that bacteria tend to attach to or become entrapped in protective sites after simple wash processing (dipping). However, most bacteria were washed out of protective sites after intermittent treatment. Direct treatment of cell suspensions with vacuum impregnation showed that it had no inactivation capacity in itself since there were no significant differences ($P \geq 0.05$) between the reduction rates of non- and vacuum impregnation treatment. These results demonstrate that the increased antimicrobial effect of vacuum impregnation can be attributed to increased accessibility of sanitizer and an enhanced washing effect in protected sites on produce. Color, texture and titratable acidity values of broccoli treated with intermittent vacuum impregnation in 2% malic acid for 30 min were not significantly ($P \geq 0.05$) different from those of untreated samples even though a storage interval was needed for titratable acidity values to be reduced to levels comparable to those of untreated controls.

1. Introduction

Fresh produce is popular worldwide because it is recognized as an important source of nutrients, vitamins and fiber for humans (Olaimat and Holley, 2012) and consumers are increasingly concerned about staying healthy through proper diet. Also, a large variety of domestic and imported produce has become available year round. For these reasons, consumption of fresh produce has increased over the past two decades (Warriner et al., 2009).

However, foodborne illness outbreaks linked to fresh produce have been on the rise due to increased consumption of fresh produce resulting in significant numbers of illnesses, hospitalizations, and deaths (Bennett et al., 2015; Callejón et al., 2015; Painter et al., 2013; Warriner et al., 2009). Among foodborne illness outbreaks, there was a *Salmonella* outbreak traced to broccoli that resulted in more than 1500 students exhibiting food poisoning symptoms in Japan in Feb., 2011 (Foodborne Illness Outbreak Database, 2014). And Taylor Farms recalled broccoli distributed to 6 states including CA, AZ, NV, OR, UT, and WA after the Washington State Department of Health detected *Listeria monocytogenes* in a random sample of product in Feb., 2011 (Food Poison Journal, 2011). Meningitis, abortion, and perinatal septicemia are the primarily manifestations of *Listeria* infection among immunocompromised individuals, pregnant women, and infants (Farber and Peterkin, 1991). Acute symptoms of *Salmonella* infection include nausea, vomiting, abdominal cramps, diarrhea, fever, and headaches (CDC, 2014). Therefore initial microbial counts before storage should be decreased by decontamination treatment in order to prevent microorganisms from reaching undesirable levels in products (Akabas and Olmez, 2007).

Washing with tap water or chlorinated water (50–200 ppm chlorine) is widely used for commercial decontamination of fresh fruits and vegetables. However, chlorine has serious drawbacks such as rapid depletion under conditions of high organic loading and formation of carcinogenic halogenated by-products generated by reaction of chlorine with organic

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matter (Wang et al., 2006). Therefore, alternative sanitizers are needed to overcome these limitations of using chlorine.

Organic acids, found in a variety of fruits and fermented foods, are one type of alternative sanitizer. They are generally recognized as safe (GRAS) and are known to have bactericidal activity (Dickson, 2006). Thus, they can be applied to inactivate foodborne pathogens on organic fresh produce. Organic acids act rapidly and kill a broad spectrum of bacteria. Moreover, they are effective within a wide temperature range and are not affected by water hardness (Marriott and Gravani, 2006).

Decontamination of produce by conventional washing and sanitizing is only marginally effective and often can only reduce numbers of pathogenic organisms by less than 2–3 log units (Gil et al., 2009; Niemira, 2012). One of the factors limiting the efficacy of washing is attachment of pathogens in inaccessible sites. Bacteria tend to locate in indentations, pores or other natural irregularities on vegetable and fruit surfaces (Sapers et al., 2008; Seo and Frank, 1999) and also attach at cut surfaces (Liao and Cooke, 2001; Sapers et al., 2008) or in cracks and punctures (Burnett et al., 2000; Sapers et al., 2008). Sapers et al. (2008) reported that there was more attachment of *Escherichia coli* in calyx and stem areas of inoculated apples than other areas of the fruit, and they could better survive in these areas than other areas of the apple surface after washing. That is, bacteria in these locations can be protected from washing or sanitizing agents. Therefore if accessibility of sanitizers into protected sites of produce is enhanced, sanitizing efficacy will be increased.

Vacuum impregnation is a technique which exchanges the internal gas or liquid of a porous product occluded in open pores for an external liquid phase. It is the action of hydrodynamic mechanisms (HDM) promoted by pressure changes (Fito et al., 1994). Two steps are needed to perform this operation after product immersion in a tank containing the liquid phase. In the first step, vacuum is applied to the system to promote the expansion and outflow of the product's internal gas. In the second step, atmospheric pressure is restored and compression leads to a great volume reduction of the remaining gas in the pores. As a result, external liquid flows subsequently into the porous structure (Fito et al., 2001). Thus, it can be a useful tool to introduce sanitizers into inaccessible sites. Therefore, the efficacy of washing with sanitizers is expected to increase by using this technique.

2. Materials and methods

2.1. Bacterial cultures and cell suspension

Three strains each of *Salmonella* Typhimurium (ATCC 19585, ATCC 43971, and DT 104), 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.

Each strain of *S.* Typhimurium, and *L. monocytogenes* was cultured in 15 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 10^8 to 10^9 CFU/ml.

2.2. Sample inoculation

Broccoli (*Brassica oleracea* L. var. *italica*) used in the evaluation was purchased from a local market (Seoul, South Korea) and stored at refrigerator temperature (4 ± 2 °C) until experiments were conducted. Broccoli florets including 1 cm of stem were cut into segments with a diameter of 4 cm. Each of the final broccoli florets were of uniform size and color without any visible decay and weight of between 8 and 9 g. We choose the dipping method to inoculate samples and needed a culture cocktail of high cell concentration because inoculation was conducted with a comparative high volume (1.5 L of peptone water (PW)). Therefore, 10 ml of culture cocktail (approximately 10^8–10^9 CFU/ml) was made as described previously and mixed with 1.5 L of 0.2% sterile PW for a final concentration of approximately 10^6–10^7 CFU/ml. Samples were completely submerged into this inoculum solution and shaken by hand for 10 min to ensure even distribution of the bacteria. After dipping, samples were separated from the cell suspension by draining the mixture on a sterilized rack and drying for 2 h in a laminar flow biosafety hood at 22 ± 2 °C to allow the attachment of bacteria, and used in each experimental trial. Three pieces of broccoli (approximately 25 g each) comprised a single set in each treatment.

2.3. Procedure of treatment

For organic acid treatment alone, inoculated samples were immersed in 1 L glass beakers containing 500 ml of 2% malic acid (99.0%; Samchun Chemical Co. Ltd., Pyeongtaek, Korea, pH 2.16) for 5, 10, 20, or 30 min at room temperature (22 ± 2 °C).

For vacuum impregnation, inoculated samples were immersed in 1 L glass beaker containing 500 ml of 2% malic acid and treated with vacuum impregnation in a vacuum oven (OV-11, JEIO TECH Co., Ltd., Daejeon, Korea) for 5, 10, 20, or 30 min at room temperature (22 ± 2 °C). A vacuum of 61.3 kPa (=8.9 psi) or 21.3 kPa (=3.1 psi) was applied to the system (atmospheric pressure is 101.3 kPa = 14.7 psi). In the experiment, two vacuum treatment methods were conducted: continuous treatment or intermittent treatment. Continuous treatment comprised of a single vacuum time interval and a single atmospheric pressure time interval. Continuous treatments consisted of 5 min treatment (comprised of 2.5 min vacuum followed by 2.5 min of atmospheric pressure), 10 min treatment (comprised of 5 min vacuum followed by 5 min atmospheric pressure), 20 min treatment (comprised of 10 min vacuum followed by 10 min atmospheric pressure), and 30 min (comprised of 15 min vacuum followed by 15 min atmospheric pressure). Intermittent treatment was made up of a collection of 5 min treatment cycles which consisted of 2.5 min vacuum treatment followed by 2.5 min atmospheric pressure treatment. Therefore, 5, 10, 20 or 30 min of intermittent treatment had 1, 2, 4, or 6 cycles, respectively. All experiments were performed using a reticulated stainless steel instrument to keep the samples submerged to prevent their being on top of the washing solution. Also, 1 ml of cell suspension was directly placed into 100 ml of treatment solution (with no broccoli sample) and treated with non-vacuum impregnation (101.3 kPa) and intermittent vacuum impregnation (21.3 kPa) as above for 30 min to ascertain if vacuum impregnation in itself has any inactivation effect on bacterial cells (1 ml of cell suspension placed into 100 ml of deionized water (DW) constituted the control). In this case, 0.7% malic acid was used instead of 2% malic acid because the antimicrobial effect of 2% malic acid with no broccoli sample was too strong to observe differences of inactivation tendency between each treatment.

2.4. Bacterial enumeration

After treatments were performed, the treated samples (25 g) and 1 ml of treatment solution containing cell suspension were immediately transferred into sterile stomacher bags (Labplas Inc., Sainte-Julie, Quebec, Canada) containing 225 ml of Dey-Engley (DE) neutralizing broth (Difco) and test tubes containing 9 ml of DE neutralizing broth, respectively. Stomacher bags containing treated samples were homogenized with a stomacher (EASY MIX, AES Chemunex, Rennes, France) for 2 min and test tubes containing treatment solution including cell suspension were mixed using a vortex mixer for 10 s. After homogenization, 1 ml aliquots of stomached samples and mixed treatment solution containing cell suspension were tenfold