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Effects of sodium hypochlorite and peroxyacetic acid on the inactivation of murine norovirus-1 in Chinese cabbage and green onion

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ABSTRACT

Chinese cabbage and green onion are important vegetables for a variety of foods in Korea. Because these vegetables are used after being briefly washed with water, the disinfecting washing needs to be applied to inactivate norovirus. We evaluate the effectiveness of sodium hypochlorite (NaOCl) and peroxyacetic acid (PAA) on inactivation of murine norovirus-1 (MNV-1) in Chinese cabbage and green onion. NaOCl treatment with 400 ppm chlorine decreased MNV-1 by more than 1 $\log_{10}$ in both samples. One minute treatment with PAA at 300 ppm reduced MNV-1 by approximately 1 $\log_{10}$ in Chinese cabbage and 1.2 $\log_{10}$ in green onion, respectively. When concentrations permissible for use on food-contact surfaces by Korea Ministry of Food and Drug Safety (MFDS) were used to disinfect the vegetables, 300 ppm of PAA was significantly ($P < 0.05$) more effective than 200 ppm of NaOCl. Although color was unchanged with higher concentrations of NaOCl and PAA, increasing the concentration of NaOCl gradually reduced hardness values and produced an unpleasant chlorinated-odor in Chinese cabbage stems. Overall, our study indicates that PAA treatment at 300 ppm for green onion or 500 ppm for Chinese cabbage is suitable for inactivating MNV-1, and does not adversely affect the food quality.

1. Introduction

Human enteric viruses including norovirus, hepatitis A virus, and rotavirus are a leading cause of foodborne viral gastroenteritis in humans worldwide (Newell et al., 2010). Of these enteric viruses, norovirus is associated with 18% of all acute gastroenteritis worldwide across all age groups (Ahmed et al., 2014). Human norovirus is primarily transmitted through fecal-oral route mediated by contaminated hands and aerosolization (Park et al., 2010). From 2007 to 2016, a total of 484 norovirus outbreaks were reported, meaning that norovirus was more prominent than pathogenic bacteria as a causative agent of foodborne diseases in South Korea. With the recent outbreaks of acute gastroenteritis in school meal services, fresh vegetables and their derivatives were pinpointed as a source for norovirus contamination (Lee, Kim, Cho, & Lee, 2012). In June 2008, a norovirus outbreak of gastroenteritis occurred at an elementary school in Incheon, South Korea, the cause of which was presumed to be dried radish salad that had been washed and steeped in water (Yu, Kim, Koh, & Lee, 2010). In April 2013, more than 400 patients with vomiting, diarrhea, and abdominal pain by ingestion of kimchi contaminated with norovirus at three schools in Jeonju, South Korea were reported to Korea Centers for Diseases Control and Prevention (Park et al., 2015).

Stable methods to propagate human norovirus (HuNoV) in cell culture have not been successful; this is despite recent attempts to cultivate HuNoV in human B cells (Jones et al., 2015) and intestinal cells (Papafragkou, Hewitt, Park, Greening, & Vinje, 2013; Takanashi et al., 2014). Thus, HuNoV research has been conducted by applying sensitive molecular techniques. However, since these techniques do not provide information to discriminate accurately HuNoV infectivity, surrogates including murine norovirus (MNV), Tulane virus, and porcine sapovirus are considered the best method for norovirus study. MNV is currently accepted as a suitable surrogate for HuNoV due to the similarities in the survival and inactivation of human norovirus (Hirneisen & Kniel, 2013).

Fresh vegetables can potentially be contaminated directly through contact by foodborne pathogens with irrigation water, organic fertilizer, and by washing (Gagné, Barrette, Savard, & Brassard, 2015). Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*) is the principal vegetable in many foods in Korea, and is used as the main ingredient for the manufacture of kimchi, kimchi stew, and fresh leaves with garlic and sesame (Lee et al., 2016). Green onion (*Allium fistulosum*) is a perennial herb and has long been used as a vegetable or as a spice in Asian countries; in particular, it is one of the most frequently used spices in Korean foods (Kim, 2010). In Korea, these two vegetables are

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mainly consumed raw or are used as ingredients of various foods after being washed simply with water. In the absence of sanitizers, although water is a useful tool for reducing potential contamination, large quantities are required to reduce microbial content (Gil, Selma, López-Gálvez, & Allende, 2009). However, there are two problems associated with this: the food industry wants to minimize water consumption (Ölmez & Kretzschmar, 2009), and norovirus persists in tap water, mineral water, and groundwater for at least two months (Ngazoa, Fliss, & Jean, 2008; Seitz et al., 2011). Due to these problems, disinfecting water with sanitizers is important in order to avoid cross contamination of fresh produce with norovirus.

Sodium hypochlorite (NaOCl) is one of the most used disinfectants due to its easy availability, cost-effective use and broad-spectrum antimicrobial activity (Gong, Wei, & Zong, 2012; Tung, Machinga, Arbogast, & Jaykus, 2013; Yap, Zilm, Briggs, Rogers, & Cathro, 2014). Because of these advantages, NaOCl (100–200 ppm) has been widely used as a disinfecting agent to reduce the pathogenic microorganisms on fresh vegetables in the fresh-cut industry (Artés-Hernández, Martínez-Hernández, Aguayo, Gómez, & Artés, 2017; Erkmen, 2010; Park, Mizan, & Ha, 2016). However, there have been many concerns that chlorination of NaOCl produces halogenated by-products, which negatively affect human health (Di Cristo, Esposito, & Leopardi, 2013; Ölmez & Kretzschmar, 2009). On the other hand, peroxyacetic acid (PAA) is a colorless agent that does not foam, and is very acidic (pH $<$ 2) (Kitis, 2004). Although it releases a strong acetic acid odor, the breakdown products (hydrogen peroxide, oxygen, and acetic acid) have low risks to health (Kingsley, Vincent, Meade, Watson, & Fan, 2014). Thus, PAA has great potential as a disinfectant alternative to chlorine.

There is a need to further evaluate antiviral effects of chemical sanitizers on fresh vegetables during the washing step. To address this, we examined the efficacy of two disinfectants (NaOCl and PAA) against MNV-1, a human norovirus surrogate, in fresh Chinese cabbage and green onion. In addition, the experiment was conducted by dividing each vegetable sample into two parts (stem and leaf of Chinese cabbage, white and green portions of green onion), in order to investigate the virucidal effect depending on the portion. Vegetable color and texture are important characteristics that impact on consumers' purchasing decisions based on freshness. Therefore, we also tested the physical and sensorial quality of food samples in order to confirm how each treatment affected their overall quality.

2. Materials and methods

2.1. Virus and cell culture

Murine norovirus strain MNV-1, which was supplied from the Department of Pathology and Immunology of Washington University, was maintained in a mouse macrophage cell line (RAW 264.7) purchased from American Type Culture Collection (ATCC, Rockville, MD, USA). We cultivated the cells per the conditions recommended for ATCC line TIB-71 (Cannon et al., 2006), with slight modification. Cells were grown in Dulbecco's minimum essential medium (DMEM; Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) at 37 °C in 5% CO_2, and then were sub-cultured every 2 days. Virus suspension was prepared as previously described with some modifications (D'Souza & Su, 2010). Cell monolayers that were 90% confluent in 150-cm^2 culture flasks were washed twice with phosphate-buffered saline (PBS, pH 7.4) after aspirating growth medium. The washed cell cultures were inoculated with a 1-mL inoculum of MNV-1, cultures were then incubated at 37 °C in 5% CO_2 for 1 h to permit virus adsorption. MNV-1 was propagated in cell cultures supplemented with maintenance medium (DMEM including 2% FBS) at 37 °C in 5% CO_2 for 3 days until cytopathic effects were observed in greater than 90% of cells. The virus-infected flasks were treated with three freeze-thaw cycles (at −70 °C and 25 °C) to release virus particles from lysed cells. The propagated MNV-1 solution was centrifuged at 2500 × *g* for 10 min at 4 °C to remove cell debris, and the supernatant containing virus was stored at −70 °C until use.

2.2. Sample preparation and inoculation

Chinese cabbages and green onions purchased from a local market (Anseong, Korea) were rinsed with deionized water to remove dirt and dust. All products were dried in a laminar flow hood for 20 min and treated with 253.7 nm ultraviolet light for 5 min each side. Stem portions (5 × 5 × 0.4 cm) and leaf portions (5 × 5 × 0.2 cm) of Chinese cabbage, and white portions (5 × 5 × 0.2 cm) and green portions (5 × 5 × 0.2 cm) of green onions were then prepared using a sterile stainless steel scissors. Individual samples were inoculated with 200 μL of MNV-1 suspension (approximate 6 $\log_{10}$ PFU/mL) and placed on a clean bench for 3 h to absorb the virus onto the surfaces of the samples.

2.3. Chemical inactivation

NaOCl (12%; chlorine ≥6%; Yakuri Pure Chemicals Co., Kyoto, Japan) at 100, 200, 300, 400, and 500 ppm of free chlorine as measured with free chlorine photometer (HI 96701; Hanna Instruments, Woonsocket, RI, USA), and PAA (32%; Sigma-Aldrich) at 50, 100, 200, 300, and 500 ppm were used as disinfectants. The virus-inoculated food samples were softly soaked into 15 mL of the manufactured disinfectant (33.3% (w/v) for Chinese cabbage stem, and 20% (w/v) for Chinese cabbage leaf and green onion) for 1 min without agitation. Control sample was treated with 15 mL of distilled water for the same length of time. When dealing with both disinfectants, all researchers wore masks and safety glasses to protect them from volatile components and unpleasant odors. The viral recovery was performed with slight modification of the method suggested by Son et al. (2014). Foods with disinfectant were then immersed in 5 mL of PBS (pH 7.4) to stop the chemical reaction with residual disinfectant. Each food sample was vortexed for 2 min and shaken at 300 rpm for 1 h to elute virus, followed by centrifugation (10,000 × *g*, 4 °C, 30 min). The supernatants were sequentially filtered using sterile 0.8 μm and 0.45 μm filters. The solution was serially diluted in DMEM and the infectious viral particles were quantified with a plaque assay.

2.4. Virus titration

Plaque assays were performed as previously described, with minor modifications (Bidawid, Malik, Adegbunrin, Sattar, & Farber, 2004). RAW 264.7 cells were seeded in 12-well plates (approximately 4×10^5 cells) and cultivated at 37 °C with 5% CO_2 atmosphere to reach 90% confluence. Each cell was inoculated with 200 μL of serially diluted MNV-1 suspension eluted from samples and incubated for viral absorption at 37 °C for 1 h. The inoculated cells were covered with 1.5 mL of 2 × type II agarose supplemented with 2 × DMEM and left at room temperature to solidify the agarose mixture. Cells were incubated for 2–3 days at 37 °C with 5% CO_2 and then fixed with 1 mL of 3.7% formaldehyde for 4 h. Formaldehyde was discarded and the overlaid agarose was carefully removed with tap water. To visualize plaques, the fixed cells were stained with 0.1% crystal violet solution for 20 min. The number of plaques was calculated and viral titers were expressed as plaque forming units (PFU)/mL. Log_{10} reduction values were calculated by following formula: $\log_{10}$ reduction = $\log_{10}$ virus titer of the control − $\log_{10}$ virus titer after treatment. Recovery percentage of MNV-1 on each sample was calculated with the following formula: % recovery = (virus titer of the control/initially inoculated virus titer) × 100.

2.5. Physical quality test: surface color and texture

The chemical-treated Chinese cabbage and green onion samples were placed in zip-lock bags (SC Johnson Co., Racine, USA) to make