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Short communication

Viral disinfection of organic fresh produce comparing Polyphenon 60 from green tea with chlorine

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ABSTRACT

Contamination of fresh produce with enteric viruses is a matter of concern because viruses may persist in food surfaces under normal storage conditions for long periods. Chlorinated compounds are the most common substances used to disinfect fresh produce but they present some disadvantages such as the formation of by-products. In this study, green tea extract (GTE), a natural compound from *Camellia sinensis*, was tested to disinfect fresh produce contaminated with 8 $\log_{10}$ of human adenoviruses (HAdV) and 6 $\log_{10}$ of infectious murine noroviruses (MNV), a surrogate of human norovirus. Chorine was also employed to compare the action of GTE with a common disinfectant. Results demonstrated that, although GTE was not as efficient as chlorine, it demonstrated good effectiveness to decrease the amount HAdV in fresh produce. Both disinfectants were more effective than just using water to reduce the load of infectious HAdV. It was also observed that both chlorine and GTE were more effective to remove HAdV than MNV, probably due to different interactions of the viruses with the plant tissues.

1. Introduction

Fruits and vegetables are part of the population's diet all over the world since they provide vitamins and minerals essential for human health maintenance. Moreover, during the last decades authorities from different countries have introduced campaigns to stimulate the consumption of fresh produce, especially among children and elderly, as they have proven to prevent coronary heart disease, hypertension, stroke, some cancers and diabetes (US Department of Health and Human Services and Department of Agriculture, 2015; WHO, 2008). However, while fresh produce help us to keep a balanced and healthy diet, they are also related to foodborne diseases as they are usually eaten raw or minimally processed and can potentially transmit pathogens (Li, Keuckelaere, & Uyttendaele, 2015). This fact, together with the increasing trend of fresh produce consumption in developed countries, the rising of food transport among continents and also the changes in food processing may have a positive correlation with the increase of reported outbreaks transmitted by fruits and vegetables (Lynch, Tauxe, & Hedberg, 2009). Moreover, there is also an upward tendency of organic fresh produce consumption, cultivated without chemical fertilizers and pesticides (De Quadros Rodrigues et al., 2014). As there are still few data about organic fresh produce, several investigations have raised concerns about the safety of this kind of food (McMahon & Wilson, 2001; Oliveira et al., 2010).

Fresh produce can be contaminated by different ways, in pre-harvest stages due to contaminated soil, water or fertilizers, or also in post-harvest procedures such as cutting, packaging and storage (Butot, Putallaz, & Sanchez, 2008). Contamination of fresh produce with enteric viruses is a matter of concern because viruses may persist in food surfaces under normal storage conditions for long periods (Rzeżutka & Cook, 2004). The pathogen most involved in produce-associated outbreaks is human norovirus (HNoV), which is responsible for 59% of the outbreaks in USA, mostly related with consumption of salads, and 53% in European Union, mostly linked with the consumption of berries (Callejón, Rodríguez-Naranjo, M. Isabel Ubeda, Hornedo-Ortega, Garcia-Parrilla, & Troncoso, 2015). In this context, prevention and hygiene measures should be taken to reduce the microbial load of fresh produce in order to decrease the risk of pathogen transmission without modifying the organoleptic properties of the produce.

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Several methods of produce disinfection have been developed, including chemical washing with chlorine, chlorine dioxide, hydrogen peroxide, irradiative treatments and treatment with ozone, among others (Goodburn & Wallace, 2013). Chlorinated compounds are the most common disinfectants used in the fresh produce industry at levels between 25 and 200 mg/L (contact time less than 2 min) as post-harvest spray or dip (FAO/WHO, 2008). However, most of the data in literature are focused in bacterial reductions achieved by chlorination, overlooking the importance of the contamination of fresh produce by enteric viruses. Another issue to take into account is the formation of by-products from chlorine such as triahalomethanes and other chemical residues (Fraisse et al., 2011; Hrudey, 2009). Hence, other substances such as plant extracts containing compounds as polyphenols, quinones, flavonoids, alkaloids, and lectins are being investigated to find alternatives to the use of chlorine to eliminate foodborne pathogens (Perumalla, Navam, & Hettiarachchy, 2011). Green tea extract (GTE) isolated from *Camellia sinensis*, for instance, has been reported to have antimicrobial effects against bacteria, fungi and viruses (Reygaert, 2014). A group of polyphenols named catechins are the compounds from GTE that have been proved to have antibacterial (Hara-kudo et al., 2005; Matsumoto, Kaihatsu, Nishino, Ogawa, & Kato, 2012) and antiviral activity (Isaacs et al., 2008; Lin et al., 2013). Recently, it has also been demonstrated the antiviral activity of catechins from GTE against feline calicivirus (FCV) and murine norovirus (MNV), (Oh et al., 2013; Seo et al., 2016), both surrogates of HNoV. Therefore, this study aimed to study the potential of GTE to be used for fresh produce disinfection. Chorine was also employed to compare the action of Polyphenon 60 from GTE with a common disinfectant. Artificially contaminated lettuce, green onions and strawberries, were treated with GTE and chlorine solutions to evaluate the inactivation of human adenoviruses (HAdV) and MNV.

2. Material and methods

2.1. Viral stocks production

HAdV type 2 (HAdV-2) stocks were produced by infecting A549 cells (permissive cells derived from human lung carcinoma). The cells were cultured in monolayers using Eagle's Minimum Essential Medium (MEM; Gibco, Carlsbad, CA, USA) supplemented with 5% foetal bovine serum (FBS) and 1 mM sodium pyruvate and incubated at 37 °C under 5% CO_2 atmosphere. Then, they were infected with HAdV-2 and, after 48 h of incubation, they were subjected to three freezing and thawing cycles to lyse the infected cells. Cell lysates were harvested and centrifuged at 1600 × *g* for 5 min to separate the viral particles from cell debris. The obtained viral suspension was titrated, aliquoted and stored at −80 °C prior to use.

MNV type 1 (MNV-1) stocks were produced by infecting RAW 264.7 cells (a macrophage-like Abelson murine leukemia virus-transformed cell line). These cells were cultured using Dulbecco Eagle's Minimum Essential Medium (DMEM; Gibco, Carlsbad, CA, USA) supplemented with 10% FBS, 1.5% HEPES, 1% non-essential amino acids and 1% L-glutamine and incubated at 37 °C under 5% CO_2 atmosphere. The procedures for cells infection and concentration of viral suspension were the same as used for HAdV-2. All viruses and cells used in his study were kindly donated by Rosina Gironès from University of Barcelona (Spain).

2.2. Fresh produce infection

Organic fresh lettuces, strawberries and green onions (with certification *Ecocert*, *IBD* and *Ecovida*) were purchased from a local store and kept at 4 °C prior to analysis. Samples were processed weighting 25 g of each fresh produce and placed in a disinfected plastic tray. Lettuces and green onions were cut into smaller pieces and strawberries were cut only when they were bigger than 2,5 cm × 2,5 cm × 2,5 cm, as recommended by the ISO/TS 15216-1:2013 (Anonymous, 2013). Then the samples were artificially inoculated as previously described (Marti & Barardi, 2016). Briefly, 100 μl containing 8 $\log_{10}$ of infectious particles of HAdV-2 or 6 $\log_{10}$ of infectious MNV-1 were uniformly distributed as small drops onto the sample surface. Finally, samples were placed in a biosecurity cabinet for approximately 1 h, until the drops dried.

2.3. Fresh produce disinfection with green tea extract

For disinfection experiments with GTE, samples previously inoculated with viruses were submerged in an aqueous solution containing 5 mg/mL or 10 mg/mL of Polyphenon 60 from green tea (Sigma-Aldrich, St. Louis, MO, USA) for 10 min. This solution contained a mixture of polyphenolic compounds from GTE, with a minimum of 60% of catechins. The concentrations of 5 mg/mL and 10 mg/mL and the period of 10 min were chosen based on previous standardization tests performed in our laboratory (data not shown). Then, samples were rinsed for 2 min in 200 mL of deionized water and the excess of water was eliminated. A sample of 25 g of fresh produce inoculated with viruses but not submitted to any disinfection procedure was included in the experiment as a control. Another inoculated sample, only rinsed with deionized water was also included as a control to discriminate the viral reduction caused by the mechanic effect of the washing.

2.4. Fresh produce disinfection using sodium hypochlorite

For experiments about disinfecting with chlorine, 2 aqueous solutions with 20 ppm and 200 ppm of free chlorine were prepared adding the corresponding concentration of a 2.5% sodium hypochlorite solution containing 2.38 g/L of free chlorine, recommended for tap water disinfection. The deionized water used to prepare disinfectant solutions had a pH 7.0 and constant temperature of 20° C. The concentration of free chlorine was always determined in the solutions using the HANNA Instruments (model HI 95711).

Samples of 25 g of fresh produce inoculated with viruses were submerged for 2 min in 200 mL of each disinfectant solution and were rinsed following the same protocol described for disinfection with GTE. The concentrations and contact time were chosen based on the suggestions from some food safety authorities, which recommends a maximum of 200 ppm of total chlorine, at pH 6.0–7.5 and contact times of 1–2 min for removal of microorganisms in washing systems (FAO/WHO, 2008; Parish et al., 2003; USFDA, 1998).

2.5. Viral elution and concentration from fresh produce

Enteric viruses were recovered from fresh produce samples following an ISO guideline (ISO/TS 15216-1:2013, Anonymous, 2013). Briefly, the samples were placed into a sterile plastic bag together with 40 mL of Tris-glycine buffer (TGBE; 100 mM Tris–HCl, 50 mM glycine and 1% beef extract, pH 9.5) and incubated at room temperature for 20 min with constant rocking (approximately 60 oscillations/min). For strawberries, 30 U of pectinase from *Aspergillus niger* (Sigma-Aldrich, St. Louis, MO, USA) was added to prevent jelly formation in the eluate. Then, the TGBE was recovered by pipetting and centrifuged at 10,000 × *g* for 30 min at 4 °C. The supernatant was transferred to a clean tube, its pH was adjusted to 7.0 (±0.5) with 1 N HCl and 0.25 vol of a 50% (w/v) polyethylene glycol (PEG) 8000/1.5 M NaCl solution were added.