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Grape seed extract for foodborne virus reduction on produce

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ABSTRACT

Grape seed extract (GSE) is reported to have antibacterial properties with few current studies on antiviral activity. Recently, we reported the effects of GSE against foodborne viral surrogates *in vitro*. This study evaluated the application of GSE (commercial Gravinol-S) against hepatitis A virus (HAV) and human norovirus surrogates, feline calicivirus (FCV-F9) and murine norovirus (MNV-1), on model produce. Washed and air-dried lettuce (3 × 3 cm^2) and jalapeno peppers (25–30 g) were inoculated with FCV-F9, MNV-1, or HAV at high (~7 log_{10} PFU/ml) or low (~5 log_{10} PFU/ml) titers, and treated with 0.25, 0.5, 1 mg/ml GSE or water for 30 s to 5 min. Treatments were stopped/diluted with cell-culture media containing 10% heat-inactivated fetal bovine serum and evaluated using plaque assays. At high titers, FCV-F9 was reduced by 2.33, 2.58, and 2.71 log_{10} PFU on lettuce; and 2.20, 2.74, and 3.05 log_{10} PFU on peppers after 1 min using 0.25, 0.50, and 1 mg/ml GSE, respectively. Low FCV-F9 titers could not be detected after 1 min at all three GSE concentrations. Low titer MNV-1 was reduced by 0.2–0.3 log_{10} PFU on lettuce and 0.8 log_{10} PFU on peppers, without reduction of high titer. GSE at 0.25–1 mg/ml after 1 min caused 0.7–1.1 and 1–1.3 log_{10} PFU reduction for high and low HAV titers, respectively on both commodities. Instrumental color analysis showed no significant differences between treated and untreated produce. GSE shows potential for foodborne viral reduction on produce as part of hurdle technologies.

1. Introduction

Hepatitis A virus (HAV) and human noroviruses (NoV) are the two epidemiologically relevant human enteric viruses that cause foodborne viral illness (Koff, 1998; Koopmans and Duizer, 2004). Foodborne viral outbreaks have been frequently traced to the consumption of contaminated fruits and raw vegetables (Berger et al., 2010). Fresh produce can become contaminated with foodborne pathogens at pre- and post-harvest. During pre-harvest, produce can get contaminated by polluted fertilizers or waste water (Cheong et al., 2009; Mara and Sleigh, 2010; Wei and Kniel, 2010). They can also get contaminated during food preparation due to cross-contamination or by infected food handlers when adequate personal hygienic practices are not followed (Barrabeig et al., 2010; Richards, 2001).

The fact that HAV and human NoV (and its surrogates) are stable on produce and food contact surfaces for weeks (Bidawid et al., 2004; Croci et al., 2002; D'Souza et al., 2006; Dawson et al., 2005; Mattison et al., 2007), increases the risk of viral outbreaks from the consumption of contaminated produce. After 28 days under refrigeration, only a 1 log reduction of HAV on spinach leaves was reported (Shieh et al., 2009). The loss of ≥2 log HAV on lettuce, fennel, and carrots was reported after storage for 9 days at 4 °C (Croci et al., 2002). It has also been demonstrated that human norovirus surrogates, such as feline calicivirus (FCV-F9), can survive for up to 7 days on lettuce and strawberries at 4 °C (Mattison et al., 2007), while bacteriophage MS2 at 4 and 8 °C are reduced by <1 log after 39 days on lettuce, carrots, cabbage, onions, peppers, tomatoes, cucumbers, raspberries and strawberries (Dawson et al., 2005). In addition, the human norovirus surrogate, murine norovirus, (MNV-1) was found to be stable across the tested pH range of 2–10, showing <1 log reduction at pH 2, whereas FCV-F9 infectivity was reduced rapidly at pH <3 and >9 (Cannon et al., 2006). Recently, MNV-1 was shown to be completely reduced after 7 days, while FCV-F9 was completely reduced after only 1 day in an orange and pomegranate juice blend at 4 °C from initial titers of 5–6 log PFU/ml (Horm and D'Souza, 2011).

This observed lengthy survival pattern of HAV and human NoV increases the need for effective decontamination methods for their removal from fresh produce. Commonly used sanitizers for produce include chlorine, chlorine dioxide, organic acids, peracetic acid, ozone, and hydrogen peroxide (Gomez-Lopez et al., 2008;

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Sapers, 2001). However, there is an increasing consumer demand for fresh vegetables that are minimally processed, with the ability to retain their nutritional and sensory properties, and that are preferably treated by natural antimicrobials to maintain safety (Francis et al., 2012). This trend has increased the interest in the use of plant-derived antimicrobials by the food industry to enhance food safety. Grape seed extract (GSE) has been shown to have antibacterial activity against both gram-negative and gram-positive foodborne bacterial pathogens (Baydar et al., 2004, 2006; Jayaprakasha et al., 2003). Our previous study showed that GSE also has antiviral effects against foodborne virus surrogates (FCV-F9; MNV-1; and MS2 bacteriophage) and HAV (Su and D'Souza, 2011a).

The objectives of this study were to determine the application of GSE for the reduction of foodborne noroviruses (using surrogates) and hepatitis A virus on model produce items (iceberg lettuce and jalapeno peppers). The overall goal was to determine the potential of using GSE as an alternative produce wash and therefore instrumental analysis to determine color changes between treated and untreated produce after storage were also undertaken. Due to the lack of cell culture methods for human NoV propagation, cultivable human NoV surrogates such as FCV-F9 (Steinmann, 2004), and MNV-1 (Wobus et al., 2004) were used to determine the effects of GSE on viral infectivity. Lettuce and jalapeno peppers inoculated with high ($\sim$7 $\log_{10}$ PFU/ml) and lower ($\sim$5 $\log_{10}$ PFU/ml) titers of FCV-F9, MNV-1, or HAV were treated with 0.5, 1, or 2 mg/ml GSE for 30 s to 5 min. The infectivity of the recovered viruses after treatment was evaluated using standardized plaque assays and compared to untreated controls.

2. Methods

2.1. Viruses and cell lines

Cell lines for the propagation of viruses included Crandell Reese Feline Kidney (CRFK) cells, RAW 264.7 cells, and fetal rhesus monkey kidney (FRhK4) cells. These cells were maintained in Dulbecco's Modified Eagle's Medium/Ham's F-12 (DMEM-F12; HyClone Laboratories, Logan, UT) supplemented with 10% heat-inactivated fetal bovine serum (FBS, HyClone Laboratories) and 1× Anti–Anti (Antibiotic-Antimycotic, Invitrogen, Grand Island, NY) at 37 °C in an atmosphere containing 5% CO_2. MNV-1 stock was obtained as a gift from Dr. Skip Virgin (University of Washington, MO).

All virus stocks were prepared as described before (D'Souza et al., 2009; Su and D'Souza, 2011a; Su et al., 2010c).

2.2. Antiviral effects of GSE

GSE, Gravinol-S, was obtained as a gift from OptiPure®, Chemco Industries (Los Angeles, CA). GSE solutions at concentrations ranging from 0.25 to 1 mg/ml in phosphate-buffered saline (pH 7.4) were filter-sterilized before use for viral treatment. Iceberg lettuce and jalapeno peppers were purchased from local grocery stores. Before inoculation with viruses, lettuce and peppers were washed with de-ionized water, treated with 70% ethanol for 1 min, washed again in sterile de-ionized water, and air-dried in a Bio-safety Level 2 (BSL-2) hood under ultraviolet light in a sterile petridish for 1 h. FCV-F9, MNV-1, or HAV (0.15 ml) at titers of $\sim$7 $\log_{10}$ PFU/ml (high viral titers) or 5 $\log_{10}$ PFU/ml (low viral titers) were individually inoculated on lettuce (approximately 3 × 3 cm^2) or pepper (weight: 25–30 g per pepper) samples to cover the surface, and allowed to air-dry at room temperature for 5 min under the BSL-2 hood. Next, 0.15 ml of 0.25, 0.5, or 1 mg/ml GSE, or water was added for 30 s to 5 min to lettuce and pepper samples that were inoculated with FCV-F9, MNV-1, or HAV. After treatments, viruses were eluted from the food surface by washing/pipetting with 0.85 ml DMEM containing 10% heat-inactivated fetal bovine serum (FBS) twice (total of $\sim$2 ml wash for viral recovery). The rich organic components present in heat-inactivated fetal bovine serum and cell culture medium are known to neutralize chemical treatments. Therefore, ten-fold serial dilutions were prepared in DMEM containing FBS and assayed for the infectivity of viruses using standardized plaque infectivity assays as described below. Viral recovery was calculated by multiplying plaque counts with the corresponding dilution factors. Titer reduction was calculated by deducting the viral titer of the GSE treated produce from the water treated produce. Each treatment was replicated thrice. Plaque assays for evaluating the infectivity of the viruses were carried out in duplicates as described below.

2.3. Infectious plaque assays

Standard procedures were followed for FCV-F9, MNV-1, and HAV plaque assays as reported earlier (Su and D'Souza, 2011a). Briefly, confluent CRFK, RAW 264.7, and FRhK4 cells were infected with 0.5 ml of FCV-F9, MNV-1, and HAV, respectively for 2 h. Viruses were then aspirated and the cells were overlaid with 2 ml complete DMEM containing 0.75% agarose and incubated at 37 °C under CO_2. After an incubation of two (for FCV-F9), three (for MNV-1) and eight days (for HAV), cell monolayers were stained with neutral red and incubated at 37 °C until plaques were visible.

2.4. Color measurements of lettuce and jalapeno peppers before and after treatment

Six lettuce leaves and six jalapeno peppers were treated with 1 mg/ml GSE for 5 min, as described above, followed by a brief rinse in water. Produce (six each) immersed in deionized water for 5 min were used as untreated controls. After each treatment, instrumental color (L*, a*, and b*, illuminant A) was measured 0–5 days post treatment using a HunterLab MiniScan XE Plus Spectrophotometer (model 45/0 LAV, 2.54-cm-diam. aperture, 10° standard observer, Hunter Associates Laboratory Inc., Reston, VA). Color changes ΔE were calculated as square root $((L1^* - L2^*)\text{-}2 + (a1^* - a2^*)\text{-}2 + (b1^* - b2^*)\text{-}2)$, where +L = Light; −L = black; +b = yellow; −b = blue; +a = red; −a = green (Robertson, 1990).

2.5. Statistical analysis

Results from the three replicate treatments and controls were statistically analyzed using ANOVA with SAS software (version 9.2, SAS Institute, Cary, NC, USA) and Tukey's test on a completely randomized design.

3. Results and discussion

Decontamination of produce with sanitizers is a critical step to reduce foodborne pathogen outbreaks and ensure food safety (Burnett and Beuchat, 2000). Recently, consumers have preferred natural antimicrobial compounds such as plant extracts over synthetic chemicals for use in food commodities that also some have health benefits, such as antioxidant, anti-inflammatory, anti-carcinogenic, and antiaging effects (Gil et al., 2000; Haslam, 1996; Nassiri-Asl and Hosseinzadeh, 2009). In the present study, the antiviral effect of GSE at 0.25, 0.5, and 1 mg/ml against human NoV surrogates (FCV-F9 and MNV-1) and HAV were evaluated on high-risk model produce (lettuce and peppers) that are frequently associated with foodborne outbreaks (Stine et al., 2005, 2011). The obtained titer reductions of the three tested viruses after GSE