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Fog, phenolic acids and UV-A light irradiation: A new antimicrobial treatment for decontamination of fresh produce

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ABSTRACT

This study evaluates synergistic interactions of food grade phenolic acids (gallic and ferulic acid) and UV-A light to achieve decontamination of fresh produce using a fog to improve dispersion of the phenolic acids on produce surface. Nonvirulent strains of *Escherichia coli* O157:H7 and *Listeria innocua* were used as model bacteria and spinach was selected as a model fresh produce. Synergistic combination of a fog deposited phenolic acid and a UV-A light treatment achieved reduction in bacterial plate count up to 2 log CFU/cm^2 independently of the initial load of the bacteria (10^4 or 10^6 CFU/cm^2). Following the treatment, fog deposited gallic and ferulic acid could be easily removed from the surface of produce by immersion in water and the treatment did not significantly alter the total endogenous phenolic content of spinach. The treatment also did not affect the texture, but impacted the color of the spinach leaves on a Hunter's Lab scale although the visual color changes were small. Overall, this technology may aid in developing alternative approaches for decontamination processes using food grade compounds.

1. Introduction

Chlorine based sanitizers are used for sanitation of wash water and preventing cross contamination of fresh produce during a washing process (Banach et al., 2015). Despite using a total chlorine concentration of 100–600 ppm in wash water (Suslow, 1997), these sanitizers can only reduce less than 2 log CFU/cm^2 of bacteria attached on the surface of fresh produce (Parish et al., 2003). This limitation results due to consumption of chlorine upon reaction with organic matter on the surface of fresh produce. Furthermore, in many cases the water used for fresh produce decontamination is refrigerated and its volume can exceed ten times the mass of fresh produce being decontaminated. This leads to extensive energy and water consumption during postharvest processing of fresh produce (Lee and Okos, 2011). Although post-harvest processing of produce using water and sanitizers is widely accepted in the industry, there is a significant unmet need to improve decontamination of fresh produce, reduce the risk of cross-contamination as well as improve process efficiency. In addition, there is a strong consumer interest in reducing the use of chemicals and processing aids. Thus, finding more sustainable alternatives to chlorine or other chemical sanitizers is highly desirable.

We previously demonstrated that the synergistic combination of gallic acid and UV-A light can inactivate more than 3 log CFU/mL of bacteria suspended in spinach wash water with a chemical oxygen demand (COD) of 2000 mg/L (Cossu et al., 2016). Another recent study showed potential of other natural compounds for inactivation of microbes based on synergistic activity with light in an aqueous environment (Luksiene and Brovko, 2013). Although inactivation of bacteria in aqueous phase can aid in sanitization of wash water, this approach is not very effective for inactivation of microbes on the fresh produce surface. This limitation could be due to limited accessibility of sanitizer to the bacteria on the surface of fresh produce as well as non-specific interactions of sanitizers with plant biopolymers and cells (Banach et al., 2015).

One of the potential approaches to improve accessibility of sanitizers to bacteria on the surface of produce is to deliver sanitizers using micron or nanoscale droplets. Using this approach, the sanitizers can be uniformly dispersed across the surface of fresh produce that may lead to improved decontamination of fresh produce. To create micron scale droplets, fogging can be an efficient approach to generate droplets that are typically less than 25 μm in size, much lower than conventional spray or mist (Matthews et al., 1995).

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The overall goal of this study was to evaluate inactivation of bacteria on the surface of spinach based on a synergistic interaction between food grade phenolic acids, i.e. gallic acid (GA) or ferulic acid (FA) deposited on the surface of produce using fog and UV-A light. GA and FA are food grade compounds (Zorn and Czermak, 2014) with a potential to limit and prevent microbial growth upon extended incubation with food (Takahashi et al., 2015; Alves et al., 2017). *Escherichia coli* O157:H7 and *Listeria innocua* were selected as model bacteria for inactivation studies. In addition to antimicrobial activity, the influence of this synergistic treatment on color, texture as well as the total phenolic content of spinach was assessed.

In summary, this study illustrates potential of a novel approach for decontamination of fresh produce using a combination of fogging approach to deposit antimicrobial compounds and subsequent exposure to UV-A light. The results of this study can aid in developing alternative approaches for decontamination processes, particularly the approaches based on using food grade compounds for fresh produce decontamination.

2. Material and methods

2.1. Bacterial cultures preparation and inoculation of spinach leaves

An avirulent strain of *Escherichia coli* O157:H7 (ATCC 700728, Manassas, VA, USA) resistant to rifampicin (Moyne et al., 2011) was cultured in tryptic soy broth (TSB) (Sigma, St. Louis, MO, USA) containing rifampicin 50 μg/mL (Fisher Scientific, Hampton, NH, USA). The Gram-positive *Listeria innocua* (ATCC 33090, Manassas, VA, USA) was cultured in the same medium. Bacteria were grown at 37 °C at 150 rpm for 18h up to a stationary phase reflecting approximately 7×10^8 CFU/mL and 1.5×10^9 CFU/mL for *E. coli* O157:H7 and *L. innocua*, respectively. Plate counting on tryptic soy agar (TSA) (Sigma, St. Louis, MO, USA) added with rifampicin 50 μg/mL confirmed the initial bacterial concentrations. Cultures were diluted in water to obtain bacterial suspensions immediately used for the antimicrobial assays.

Ten microliters from a 1×10^8 CFU/mL or 1×10^6 CFU/mL bacterial suspension were deposited on a square sample of baby spinach (*Spinacia oleracea)* cut leaves of 1 × 1 cm to obtain an inoculum of 1×10^6 CFU/cm^2 or 1×10^4 CFU/cm^2 on the spinach surface area. Inoculum was spread with a sterile tip on the surface of the leaf sample and allowed to dry for 20 min at room temperature.

2.2. Exposure of inoculated spinach leaves to fog and UV-A light

A fog producing machine (Hurricane 700, Chauvetdj, Sunrise, FL, USA) was connected through a 5 cm diameter hole on the wall of a 30 × 40 × 55 cm plastic box. Solutions of gallic acid (GA) (40 mM) or saturated ferulic acid (FA) (24 mM) were prepared in water containing 1% w/v glycerol. Inoculated spinach samples were incubated for 10 min in the chamber, where fog was produced by pumping 10 mL of either phenolic acids solutions in the device.

After this, the samples were immediately transferred to a photoreactor consisting of four UV-A light fluorescent bulbs (320–400 nm, peak wavelength 360 nm, 18 Watt, Actinic BL, Philips, Holland) mounted on the ceiling of a box producing an UV-A light intensity of 2.6 ± 0.2 mW/cm^2 at 8 cm from the lamps measured with a radiometer (UV-340A, Lutron, Taipei, Taiwan). Samples were exposed to UV-A light for 0, 10 or 30 min irradiation. Following this, leaves inoculated with 1×10^6 CFU/cm^2 were then inserted in a tube containing 10 mL of PBS and vortexed for 10 s to remove the bacteria from the treated sample leaf. Samples inoculated with 1×10^4 CFU/cm^2 were inserted in an eppendorf tube containing 1 mL of PBS and vortexed as above. Dilution of the suspensions containing bacteria were then plated on TSA added with rifampicin for plate counting after 24 h incubation at 37 °C. Controls consisted of inoculated leaves that were (a) incubated with fog but not exposed to UV-A light, (b) exposed to UV-A light but not to fog and (c) exposed to neither fog nor UV-A light.

2.3. Measurement of phenolic acid deposition and endogenous phenolic content of phenolic acid fog/UV-A light treated spinach leaves

Spinach samples of 2 × 1 cm size were incubated in fog and exposed to UV-A light as described above. These samples were inserted into an eppendorf tube with 1 mL of water and vortexed to wash the leaf and resuspend the deposited phenolic acid. A volume of 500 μL of the washing suspension was then added with 200 μL of a 1:10 dilution (v/v in water) of Folin & Ciocalteu's phenol reagent (Sigma, St. Louis, MO, USA) and 100 μL of 1M Na_2CO_3. A 100 μL aliquot was placed within a well of a 96-well plate and the absorbance at 750 nm of the solution was measured using a Spectramax340 device (Molecular Devices LLC, Sunnyvale, CA, USA). A standard curve made with gallic acid equivalent (GAE) in μg/mL was also performed to determine the concentration of the washed phenolic acid from the leaf as μg/cm^2 of GAE. Controls of fog with water or phenolic acid fog treated leaf without exposure to UV-A light were also performed.

Spinach leaf samples of 2 × 1 cm size incubated with fog and with or without exposure to UV-A light were washed in 1 mL of water to remove GA or FA deposited on the leaf surface and then transferred to a new tube containing 1 mL of acidified methanol (1% concentrated HCl (37%) in methanol), vortexed and sonicated in a sonication bath for 10 min. A volume of 500 μL of the extraction solution was then evaporated under vacuum and the pellet was reconstituted in 100 μL of water. The entire volume of 100 μL was then added with 40 μL of a 1:10 dilution (v/v in water) of Folin & Ciocalteu's phenol reagent and 20 μL of 1M Na_2CO_3. The absorbance of the solution at 750 nm was then measured as above. The total phenolic content of the leaf sample was measured as μg/cm^2 of GAE.

2.4. Texture and color analysis of phenolic acid fog/UV-A light treated spinach leaves

Fog/UV-A light treated square samples of 3 × 3 cm were analyzed for resistance to incident force with a TA-TXPlus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA) with texture resistance expressed as peak of compression force (N) at 2 mm for 2 s.

Samples were also analyzed for color with a ColorFlex EZ Spectrophotometer (Hunter Lab, Reston, VA, USA). Hunter's color values, including lightness (L), redness (a), and yellowness (b), were analyzed by the EasymatchQC software (Hunter Lab, Reston, VA, USA). A total color change (ΔE) of 2.3 was considered as just noticeable difference (JND) (Sharma and Bala, 2002).

2.5. Statistical analyses

All experiments were performed in three biological replicates. The mean of the triplicates is shown plus the standard deviation. Student's *t*-test was used to assess the significance at 95% confidence interval.

3. Results and discussion

3.1. Antimicrobial effect of combined treatment on spinach leaf

Fig. 1 (Fig. 1) illustrates the antimicrobial activity of fog deposited GA or FA on spinach leaves in combination with UV-A light. In case of bacteria inoculated at 1×10^6 CFU/cm^2 levels, approximately 1.5 log reductions were observed for either of the bacterial strains after 10 min of UV-A light exposure following deposition of GA or FA on leaf surfaces ($p < 0.05$). With extended UV-A light exposure of 30 min, no significant additional bacterial reduction was achieved for a combination of the selected phenolic acids and UV-A light treatment. The controls for this set of experiments included incubation of the inoculated leaves under UV-A light; incubation of inoculated leaves with phenolic acids