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Antimicrobial effect of synergistic interaction between UV-A light and gallic acid against *Escherichia coli* O157:H7 in fresh produce wash water and biofilm

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ABSTRACT

A synergistic interaction between gallic acid (GA) and UV-A light (UV-A + GA) to inactivate *E. coli* O157:H7 in spinach wash water and in biofilm was evaluated. A 30-min exposure to UV-A light in presence of 10 mM GA had relevant biological effect in the inactivation of *E. coli* O157:H7 in suspension in the absence (>5 log(CFU/mL)) and the presence of organic content (>3-log(CFU/mL) in 2000 mg O_2/L COD (Chemical Oxygen Demand) organic load), and resulted in ~80% decrease in the metabolic activity of *E. coli* O157:H7 biofilm. GA solutions could be recycled through at least 3-cycles of UV-A treatment without a significant loss in antibacterial effect. Catalase reduced the extent of *E. coli* O157:H7 inactivation from the UV-A + GA treatment suggesting that generation of hydrogen peroxide was partially responsible for the observed antimicrobial effect. The UV-A + GA treatment was also found to be effective in causing >3 log(CFU/mL) reductions in *E. coli* O157:H7 on the surface of spinach leaves. UV-A + GA treatment can serve as an effective intervention in the fresh produce sanitation.
Industrial relevance: The results of this study show that a synergistic interaction between gallic acid (GA) and UV-A (365 nm) light is an effective treatment for sanitation of fresh produce and water used to wash fresh produce. It was also found to be effective against the *E. coli* O157:H7 biofilm. The attractive attributes of this technology include a relatively low cost; specific, light-triggered activity; non-toxic nature and scalability. Thus, this technology has potential to replace conventional chemical sanitizer-based sanitation approaches.

1. Introduction

Various technologies are used in the food industry for the sanitation of food processing equipment and food materials, particularly fresh produce. One of the key goals in the sanitation processes is to limit the cross-contamination between food materials and contaminated equipment or vice-versa. Microbial cross-contamination has been identified as one of the leading factors that can lead to nationwide outbreaks of foodborne illnesses. To limit cross-contamination, both physical methods such as pulsed electric fields, pulsed light and sonication; (US FDA, 2015a) and chemical methodologies such as chlorine- or peroxide-based sanitizers (Virto, Mañas, Álvarez, Condon, & Raso, 2005) have been proposed. The chemical sanitizers are often preferred for the sanitation of process water and equipment due to cost effectiveness, large scale of process equipment and ease of implementation and control. Despite their significant advantages, the key limitations of the several chemical sanitizers include: (a) limited efficiency in the presence of organic content (Van Haute, Sampers, Holvoet, & Uyttendaele, 2013), (b) production of harmful by-product (Nieuwenhuijsen, Toledano, & Elliott, 2000) and (c) the absence of a regeneration cycle to enable re-use of the added sanitizer.

Although photosensitizers (PS) are widely used for the sanitation in biomedical field (Luksiene & Brovko, 2013), their application to sanitize process water used during food processing has been only recently introduced (Cossu, Ercan, Tikekar, & Nitin, 2016; Ercan, Cossu, Nitin, & Tikekar, 2016). The generation of reactive oxygen species (ROS) by PS upon exposure to ultraviolet (UV) or visible (VIS) light and the subsequent killing of microorganisms can provide an effective alternative to address some of the key limitations of chemical sanitizers. Recent studies have demonstrated the effectiveness of food-grade photosensitizers with the synergistic effect of visible light (Cossu et al., 2016) or UV-A

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light (Ercan et al., 2016) for the treatment of wash water. These prior studies focused on the use of food-grade dyes or inorganic particles as PS agents for the inactivation of viral and bacterial targets in the presence of organic content in wash water. The agents selected in these prior studies have a restrictive use in food applications.

In this study, we evaluated the enhanced antimicrobial activity through a synergistic interaction between gallic acid (GA) and UV-A (340–400 nm) (referred to as UV-A + GA treatment henceforth) light against planktonic bacteria and bacteria in a biofilm model in the presence of wash water containing added organic matter from spinach. GA is a phenolic compound naturally present in food matrices such as tea leaves and spices like sumac, produced after the hydrolysis of an ester bond in gallated catechins (Narumi et al., 2014). GA is a generally recognized as safe (GRAS) compound (US FDA, 2015b). Thus, a successful application of GA as an effective sanitizer will provide a food-grade antimicrobial compound to address a critical challenge of microbial cross-contamination. Although GA is known to act as a mild antimicrobial agent by itself (Díaz-Gómez, Toledo-Araya, López-Solís, & Obreque-Slier, 2014), little is known about its enhanced antimicrobial activity upon exposure to UV-A radiation. A previous study evaluated antimicrobial activity of 4 mM GA upon exposure to 400 nm blue light emitting diode (LED) light and observed that >5-log reduction in *Staphylococcus aureus* could be achieved after 15 min of exposure without the presence of organic content. They attributed this enhanced antimicrobial activity of GA to lipid oxidation of bacterial cell membrane from ROS such as hydrogen peroxide and hydroxyl radicals generated from exposure of GA to LED light (Nakamura et al., 2012). Another recent study by the same group evaluated LED light enhanced antibacterial activity of diverse phenolic compounds including GA on Gram-positive and Gram-negative bacteria and found that although the treatment was effective on both types of bacteria, Gram-positive strains were more resistant to inactivation (Nakamura et al., 2015). Nevertheless, the literature lacks a systematic study involving (a) ability of UV-A light to enhance the antibacterial activity of GA; (b) efficacy of the proposed process in the presence of realistic levels of organic load in wash water form food processing industry, (c) ability of UV-A + GA treatment to inactivate biofilms, and (d) understanding of the mechanisms responsible for the observed enhancement of antimicrobial activity by synergistic interaction between UV-A and GA. This study attempts to address each of these gaps in the literature.

We evaluated the antimicrobial effect of UV-A + GA treatment on *Escherichia coli* O157:H7 in simulated wash water and as a biofilm. We also investigated the re-usability of GA for this process after multiple cycles of sanitation processes, and evaluated its mechanism of action. In addition, we also examined the localization of GA at the cellular level. A successful demonstration of the effectiveness of UV-A + GA treatment as an antimicrobial agent will offer new opportunities for the food industry to introduce sanitation systems based on GRAS compounds and address the existing challenges pertaining to sanitation.

2. Material and methods

2.1. UV-A light device

A bench-top, batch type equipment was used for the UV-A light treatment. The equipment consisted of four UV-A light fluorescent bulbs (320–400 nm, peak wavelength 360 nm, 18 W, Actinic BL, Royal Philips, the Netherlands) mounted on the ceiling of a closed box. A more detailed description of the system is reported in a previous study (Cossu et al., 2016). The intensity of the UV-A light applied to the surface of the sample placed in the equipment was 2646 ± 212 μW/cm^2 as measured with the radiometer model UV-340 A (Lutron, Taipei, Taiwan).

2.2. Bacterial culture

Escherichia coli O157:H7 (ATCC 700728, Manassas, VA) was a gift from Prof. Linda Harris at the University of California-Davis. The bacterium has been depleted of two shiga toxin-like genes (*stx1* and *stx2*) and is rifampicin-resistant (Moyne et al., 2011). The bacterium was cultured in lysogenic broth (LB) (casein peptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L), added with rifampicin 50 μg/mL or in LB agar with the same antibiotic (Fisher Scientific, Hampton, NH, USA). Bacteria were grown at 37 °C at 250 rpm until an absorbance at 600 nm of 1.5, reflecting a stationary phase, was reached (approximately 7×10^8 CFU/mL).

2.3. Preparation of clarified spinach homogenate and COD measurement

Spinach (*Spinacia oleracea)* leaves were bought from a local retail store. Ten gram of spinach were added to 90 mL of sterile MilliQ water and blended at high speed in a blender (Simple Blend™ 100 Blender, Oster®, USA) with 2 pulses of 30 s each. Homogenate was recovered and transferred to 50-mL conical tubes for a following centrifugation at 13,000 ×*g* for 10 min. Supernatant was recovered and centrifuged an additional time as above. The resulting supernatant was diluted and analyzed for the chemical oxygen demand (COD) using high range COD digestion vials (Hach, Loveland, CO, USA) as already described (Cossu et al., 2016). A resulting COD content of 2800 mg/L O_2 was measured. The supernatant was appropriately diluted in sterile water for the subsequent experiments involving microbial reduction in the presence of varying organic loads.

2.4. Inactivation of Escherichia coli O157:H7 by synergistic interaction between UV-A light and GA (UV-A + GA) treatment

A culture of *E. coli* with an A_{600} nm = 1.5 OD was diluted in varying amounts of organic loads derived from the blended spinach suspension (0, 500 and 2000 mg/L COD) to approximately 2×10^6 CFU/mL. One milliliter of the bacterial suspension was added to 1 mL of gallic acid (GA) (Sigma, St. Louis, MO, USA) solutions with identical organic load to achieve 0, 1, 5 or 10 mM of GA concentrations. The volume containing 1×10^6 CFU/mL was placed within a well of a 12-well flat bottom polystyrene plate (Corning, Corning, NY, USA) and exposed to UV-A light in the photo-reactor at a distance of 8 cm from the light source for 0, 5, 15 and 30 min. An aliquot of the sample was withdrawn for each time point, and serially diluted in phosphate-buffered saline (PBS) (USB Corporation, Cleveland, OH, USA). Aliquots of 100 μL were plated on LB agar added with rifampicin 50 μg/mL for the bacterial count. Plates were incubated at 37 °C for 24 h and the population of *E. coli* was interpreted in terms of log(CFU/mL) sample. Control experiments consisting of bacteria incubated at room temperature for up to 1 h either in the presence of GA but without UV-A light or in the absence of GA but in the presence of UV-A light were also performed. Moreover, as a control to see the effect of pH on treatment, the pH of DI water was adjusted to 3.0 with HCl and mixed with bacteria to reach 1×10^6 CFU/mL population. Then, the reductions of the bacteria population under UV-A and in the dark were observed.

2.5. Mathematical modeling of inactivation of Escherichia coli O157:H7

The Weibull model was used to describe the inactivation by UV-A and UV-A+GA treatments. This model includes nonlinearity of semi-logarithmic survivor curves and the classical first-order approach (Van Boekel, 2002).

$$\log_{10}\frac{N}{N_0} = \frac{1}{2.303}\left(\frac{t}{\alpha}\right)^{\beta} \quad (1)$$