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Antimicrobial activity of curcumin in combination with light against *Escherichia coli* O157:H7 and *Listeria innocua*: Applications for fresh produce sanitation

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ABSTRACT

Sanitation of fresh produce is critical for reducing microbial load and preventing the risk of foodborne illness. This study evaluated an alternative approach for the inactivation of bacteria in wash water and fresh produce using curcumin in combination with UV-A or visible light. This study characterized the inactivation of *Escherichia coli* O157:H7 and *Listeria innocua* under different experimental conditions, such as curcumin concentration, time of light exposure, incubation temperature, pH and chemical oxygen demand content (COD). The combination of UV-A light and curcumin was also evaluated to prevent cross-contamination of fresh produce from contaminated wash water during simulated spinach and tomato washing. It was observed that UV-A light in combination with low concentrations of curcumin (1–10 mg L^{-1}) was able to inactivate more than 5 log CFU mL^{-1} of *E. coli* O157:H7 and *L. innocua* in solution regardless of the incubation temperature. In addition, reduced pH increased the antimicrobial activity of curcumin in combination with light against both *E. coli* O157:H7 and *L. innocua*. This enhancement in antimicrobial activity reduced the time required for the inactivation of 5 log CFU mL^{-1} of *E. coli* O157:H7 from 10 min to 2 min of treatment. Combination of curcumin with UV-A light was more effective than its combination with visible light for achieving the same level of reduction in bacterial plate count. Although the presence of COD in simulated wash water decreased the antimicrobial photo-activity of curcumin, more than 5 log CFU mL^{-1} of bacteria could still be inactivated in the presence of high COD (1000 mg L^{-1}). Furthermore, the combination of UV-A light and curcumin was able to significantly inhibit bacterial cross-contamination from contaminated wash water to produce surfaces. In summary, the results demonstrate significant potential of curcumin in combination with light for the inactivation of bacteria in food systems including sanitation of wash water and fresh produce.

1. Introduction

Over the recent years, many foodborne outbreaks could be traced back to consumption of contaminated fresh produce (Callejón et al., 2015). Since significant fraction of fresh produce is consumed raw, sanitation of fresh produce is extremely important for reducing the risk of food borne illness. Peracetic acid and chlorine-based sanitizers are commonly used for the sanitation of fresh produce (Fatica, 2009). However, the antimicrobial activity of these common sanitizers can be reduced by non-specific reactions with dissolved organic content. Due to this limitation, high concentration of sanitizers (100–300 mg L^{-1}) are commonly applied in the fresh produce industry to achieve an active chlorine concentration of 5–15 mg L^{-1} (Hassenberg et al., 2017; Pan and Nakano, 2014). This practice of using high concentrations of chemical sanitizers have raised concerns regarding the possible generation of toxic halogenated by-products due to hyper-chlorination of wash water and the impact these toxic by-products could have on human health and the environment (Gil et al., 2009).

Antimicrobial alternatives using food grade compounds can potentially address some of the key challenges with conventional sanitizers.

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Curcumin is a phytochemical polyphenolic compound found on Turmeric (*Curcuma longa*) rhizomes that has been traditionally used in the food industry as a natural colorant due to its vibrant yellow color or as a dietary supplement (Ammon and Wahl, 1991; Hatcher et al., 2008; Pandey et al., 2010; Saldanha et al., 2016). Recently, studies have reported the application of enhanced antimicrobial activity of curcumin in combination with light for few food systems such as oysters and maize kernels (Binbin et al., 2016; Liu et al., 2016; Temba et al., 2016; Tosati et al., 2018; Wu et al.,2016). Enhancement in antimicrobial activity of curcumin in the presence of light radiation is attributed to the photo-excitation of curcumin (Haukvik et al., 2009). This photo-excitation of curcumin can result in the formation of reactive oxygen species (ROS), such as singlet oxygen species, which can inactivate microbes via oxidative damage. Recent studies have also indicated that, in addition to ROS, the photo-excitation of curcumin can generate curcumin radicals which can cause significant DNA damage (Nafisi et al., 2009; Qian et al., 2016). Similar to curcumin, our research group and others have demonstrated that the combination of certain food grade antimicrobial compounds such as gallic acid with light radiation can present a synergistic antimicrobial activity (Cossu et al., 2016; Horbury et al., 2016; Shirai et al., 2015, 2017). Despite significant potential, relatively high concentration levels of natural antimicrobials (1000–10,000 mg L^{-1}) and long light exposure times (15–30 min) required for significant antimicrobial activity may limit the translation of these innovative antimicrobial solutions for food applications. In addition, the synergistic antimicrobial activity of some of these compounds can be significantly reduced at refrigerated temperature, which could limit their application to the sanitation of fresh produce (Oliveira et al., 2017).

To address these potential challenges, we proposed the combination of low concentrations of curcumin (0–10 mg L^{-1}) and UV-A light as an alternative antimicrobial approach for fresh produce sanitation. The antimicrobial activity of light-exposed curcumin against *Escherichia coli* O157:H7 and *Listeria innocua* was assessed under a range of experimental conditions, such as different curcumin concentrations, time of light exposure, wavelength of light, incubation temperature and pH. In order to evaluate the applicability of this approach for fresh produce sanitation, the antimicrobial photo-activity of curcumin was evaluated in simulated wash water with different levels of chemical oxygen demand (COD). Furthermore, the potential of the combination of UV-A light and curcumin to prevent bacterial cross-contamination from wash water to produce surface was assessed during simulated washing of spinach and cherry tomatoes.

In summary, the key novel aspects of this study are: (a) combination of curcumin and light for the sanitation of wash water for fresh produce applications; (b) illustrate the role of pH in influencing synergistic antimicrobial activity of curcumin with light; and (c) characterize the influence of process variables on synergistic antimicrobial activity of curcumin in combination with light. Addressing these questions will illustrate the potential of applying the synergistic combination of curcumin and light for addressing key challenges in fresh produce sanitation.

2. Materials and methods

2.1. Reagents

Curcumin (> 95%), Ethanol (99%), Citric acid and Sodium citrate were obtained from Sigma-Aldrich (St. Louis, MO, USA). Lysogeny broth (LB), Tryptic Soy Broth (TSB), Tryptic Soy Agar (TSA) and Phosphate-buffered saline (PBS) were purchased from Fisher BioReagents (Pittsburgh, PA, USA). Fresh spinach leaves (*Spinacia oleracea*) and cherry tomato (*Solanum lycopersicum* var. *cerasiforme*) were purchased from a local retail store and stored at 4 °C (up to one week) until further use. Ultrapure water was obtained using a Milli-Q filtration system (EDM Millipore; Billerica, MA, USA).

2.2. Microbial culture

Escherichia coli O157:H7 (ATCC 700728, Manassas, VA, USA) and *Listeria innocua* (ATCC 33090, Manassas, VA, USA) were provided by Dr Linda Harris and Dr Trevor Suslow, respectively, from the Department of Food Science and Technology at University of California, Davis. Both bacterial strains have been modified with a Rifampicin resistant plasmid. Bacteria were grown overnight to the stationary phase in TSB broth containing Rifampicin 50 mg L^{-1} at 37 °C and 150 rpm. The concentrations of the stationary phase overnight-grown bacteria were approximately 2.0×10^9 CFU mL^{-1}, as determined by the standard plating counting method.

Fresh bacterial suspensions of *E. coli* O157:H7 and *L. innocua* were prepared before each experiment and centrifuged at 10,000*g* for 10 min in order to wash the bacterial pellet before resuspension in sterile water and dilution to a final concentration of 2.0×10^6 CFU mL^{-1}.

2.3. Curcumin solutions

Stock solution of curcumin (20 mM; 7367 mg L^{-1}) was prepared in ethanol (99% v/v) and stored in the dark at 4 °C until further use. Fresh curcumin solutions were prepared before each experiment by serially diluting aliquots of the stock solution with sterile water to final curcumin concentrations of 2, 10, 20 and 100 mg L^{-1}. The curcumin stock solution was also diluted using citrate buffers (100 mM) in order to achieve a final curcumin concentration of 10 mg L^{-1} at different pH's, ranging from 2.5 to 5.0.

2.4. Light source

The light source consisted of four UV-A lamps (320–400 nm; 18 W; Actinic BL, Philips, Holland) located on the inside-top of a plastic chamber. Samples were positioned at the center of the chamber, 8 cm away from the lamps. The average UV-A light intensity at the center of the chamber was 32 W m^{-2}. In addition to UV-A light, a set of experiments were also performed using four cool white lamps (400 nm–800 nm; 15 W; T8 Cool White, Philips, Holland). The average visible light intensity of the cool white lamps was 67 W m^{-2}.

2.5. UV-A light irradiation of curcumin-treated E. coli O157:H7 and L. innocua

The antimicrobial activity of curcumin under UV-A light radiation was evaluated against *E. coli* O157:H7 and *L. innocua*. Prior to UV-A light exposure, curcumin-treated bacterial samples were prepared by mixing one milliliter of fresh *E. coli* O157:H7 or *L. innocua* suspensions with one milliliter of aqueous solutions of curcumin, achieving a final bacteria concentration of 1.0×10^6 CFU mL^{-1} and final curcumin concentrations of 1, 5, 10 and 50 mg L^{-1}. After mixing curcumin and bacterial suspensions, the maximum ethanol concentration in the samples was less than 1% (v/v). The prepared curcumin-treated bacterial samples were incubated in the dark for 5 min before light exposure.

UV-A light exposure of the bacterial samples were performed as previously reported (Oliveira et al., 2017). Briefly, two milliliters of curcumin-treated bacterial samples were placed in an individual well of a sterile 12-well flat bottom polystyrene plate. The plate was positioned at the center of the UV-A light chamber. The samples were exposed to UV-A light for 5 min. After exposure, the samples were serially diluted in sterile PBS and spread on TSA plates, which were then incubated for up to 48 h at 37 °C before bacterial colonies were enumerated using the standard plate counting method. Bacterial populations were determined and expressed as colony forming unit per milliliter (CFU mL^{-1}). Curcumin-treated bacterial samples not exposed to UV-A light and curcumin-free bacterial samples exposed to UV-A light were the controls for this set of experiments.