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Disinfection of selected vegetables under nonthermal treatments: Chlorine, acid citric, ultraviolet light and ozone

Daniela Bermúdez-Aguirre*, Gustavo V. Barbosa-Cánovas

Center for Nonthermal Processing of Food, Washington State University, Pullman, WA 99164-6120, USA

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ABSTRACT

Lettuce, tomatoes and carrots were evaluated under four disinfection methods. Chlorine (50, 100 and 200 ppm) was compared for effectiveness with citric acid (0.5, 1 and 1.5%), ultraviolet light (UV-C) (0.65 and 1.6 mW/cm^2) and ozone (5 ppm) to inactivate *Escherichia coli* ATCC 11775. Processing times were from 3 min up to 60 min. Hunter color parameters, color functions (ΔE, *hue, chroma*), tomato color index (TCI) and whiteness index (WI) were evaluated after disinfection. Results showed that citric acid was not effective for inactivation of *E. coli* at the tested conditions. UV-C was effective in the inactivation of the microorganism when fluence was higher, being more effective in the smooth surface of tomato (2.7 log). Meanwhile, ozone was also able to inactivate bacteria in tomatoes (2.2 log) after only 3 min. Carrots and lettuce showed lower inactivation for all treatments because of their porous and roughened surfaces. UV-C was the treatment that most affected the color of the produce; it generated browning of lettuce, and increase of TCI and WI of carrots. Ozone also affected the greenness of lettuce. Concentration, dose and processing times of novel disinfection methods need to be evaluated not only for microbial counts, but also sensory properties.

1. Introduction

The economic cost related with foodborne illnesses in the United States is higher than 50 billion of dollars per year involving more than 48 millions of persons (Scharff, 2012). Some of the pathogenic microorganisms found in fresh produce and associated with these foodborne outbreaks are *Escherichia coli, Salmonella, Shigella* spp. and *Listeria monocytogenes* (Park, Alexander, Taylor, Costa, & Kang, 2008). Several recalls in the United States have been issued in 2011 involving the presence of *E. coli* O157:H7 in romaine lettuce and *Salmonella* in grape tomatoes and carrots, sold as separate units or as a part of pre-packaged salads, involving the entire country (FDA, 2012). *E. coli* has shown the ability to attach very strongly to leafy structures, which makes it difficult to remove the cells from fresh produce (López-Gálvez, Allende, Selma, & Gil, 2009).

Some chemicals have been evaluated for use as disinfectant agents in produce, such as chlorinated water and chlorine dioxide (Park et al., 2008). However, the use of chlorine has been associated with the formation of carcinogenic compounds in the last few years, and some pathogens have been shown to be more resistant to the lethal action of these compounds (Allende, Selma, López-Gálvez, Villaescusa, & Gil, 2008). There is a current need to provide fresh and microbiologically safe fresh-cut produce for consumers (Allende, Tomás-Barberán, & Gil, 2006; Park et al., 2008). Also, there has been an important increase in the sale of fresh produce in the last several years because of consumers' trend to eat healthy food (Thilmany et al., 2007). Thus, new sanitizers or technologies to disinfect fruits and vegetables should be efficient in the inactivation of pathogens while maintaining the sensory quality of the product (Allende et al., 2008).

Some of the novel technologies of disinfection methods in food include the use of ultraviolet light, ultrasound, ozone, irradiation, cold plasma, and organic acids, among others. Ultraviolet light-C ($100 < \lambda < 280$ nm) is able to inactivate microorganisms because of the irreversible damage to DNA (Otto et al., 2011). It has been used to disinfect water, air, surfaces, containers and vegetable commodities (Allende et al., 2006). Ozone has been approved in the United States to be used in gaseous or liquid phase as a disinfectant of food because it has better antimicrobial properties than chlorine (Kim, Yousef, & Khadre, 2003). Ozone is commonly used to disinfect drinking water; however, one of the main challenges of ozone as a sanitizer is the poor stability when organic matter is present (Selma, Allende, López-Gálvez, Conesa, & Gil, 2008). Meanwhile,

* Corresponding author. Tel.: +1 509 335 8109; fax: +1 509 335 2722.
E-mail address: daniela@wsu.edu (D. Bermúdez-Aguirre).

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organic acids are used in the food industry to extend the shelf-life of some products, but when used in higher concentrations these acids are able to inactivate microorganisms because of the acidification of the cytoplasm of the bacterial cell (Virto, Sanz, Álvarez, Condón, & Raso, 2005). Some of the organic compounds used for disinfection are propionic, acetic, malic, citric, lactic, and tartaric acid, among others (Huang & Chen, 2011; Rahman, Jin, & Oh, 2010; Sagong et al., 2011).

The aim of this work was to evaluate the use of three nonthermal treatments in the inactivation of a surrogate microorganism of the pathogenic *E. coli* O157:H7 in fresh produce and to compare them with the conventional chlorination process. Cells of the non-pathogenic *E. coli* ATCC 11775 were used and inoculated in three challenge surfaces: carrots, tomatoes and lettuce and were exposed to ultraviolet light, ozone and citric acid solutions during certain disinfection times.

2. Materials and methods

2.1. Vegetable samples

Three different samples were chosen to study the degree of disinfection: a green leafy product such as romaine lettuce (*Lactuca sativa* L. var. *longifolia)*, a smoothed surface fruit such as grape tomatoes (*Lycopersicon lycopersicum)* and a porous surface vegetable such as baby carrots (*Daucus carota* L.). All of the vegetables were purchased in a local supermarket and kept under refrigerated conditions (4 °C) until used. Initial mesophilic loads were evaluated for each vegetable.

2.2. E. coli ATCC 11775

2.2.1. Growth

A strain of *E. coli* ATCC 11775 was used as a surrogate microorganism of *E. coli* O157:H7 because of its higher resistance (Gurtler, Rivera, Zhang, & Geveke, 2010). *E. coli* ATCC 11775 was rehydrated with 5 ml of sterile nutrient broth (Bacto: Becton, Dickinson and Co., Sparks, MD). After 30 min the cell suspension was inoculated into 100 ml of sterile nutrient broth and incubated at 35 °C with continuous agitation at 225 rpm in an orbital shaker. Absorbance was read at 564 nm in a 8452A diode array spectrophotometer (Hewlett-Packard, Palo Alto, CA) until reaching the early stationery phase, approximately after 17 h. Bacterial growth was assessed using pour-plate enumeration for *E. coli* cells. One ml of the early stationery phase plus one ml of a glycerol solution (20 ml glycerol/100 ml sterile water) were mixed together and stored at −21 °C.

2.2.2. Inoculation of vegetables

For inoculation of vegetables, samples were prepared as follows. All vegetables were rinsed with sterile water to remove some of the natural flora (only for *E. coli* experiments) and any other matter (e.g. soil) before treatment. For lettuce, two to three outer leaves were discarded and the internal leaves were cut with a sterile knife into small pieces of approximately 5 × 5 cm with a weight of about 1 g (±0.15 g). Carrots were cut in small discs with another sterile knife (approximately 1 g ± 0.23 g). Both vegetables needed to be cut to fit the treatment chamber of the ozone equipment. Grape tomatoes were left whole (9.48 g ± 1.01). For infection of the produce, the stock culture was added to 100 ml of sterile nutrient broth, agitated by hand for few seconds and then placed in an orbital shaker at 37 °C (218 rpm) for 17 h. The next day, 0.5 ml of the culture were added to 500 ml of sterile water and vegetable samples were left in direct contact with the microbial solution (10^7 cfu/ml) for 30 min. After that, samples were dried in a laminar hood for 30 min to fix the bacteria on the surface of the product; this time allowed to have a high initial microbial load and was in agreement with the reported by Lang, Harris, and Beuchat (2004); Yaun, Sumner, Eifert, and Marcy (2004) and Sapers and Jones (2006). Afterward, samples were transferred aseptically to the different solutions or treatments to start the disinfection process. Several samples of each vegetable after the exposition with *E. coli* cells were taken directly to evaluate the time zero for each sample.

2.2.3. Microbiological analysis

Approximately 1 g of each vegetable (for lettuce and carrots) and a whole tomato were placed into 9 ml and 90 ml of sterile peptone water (0.1%), respectively, inside a sterile plastic bag and homogenized with a Seward 400 Circulator Stomacher (Seward, Ltd., London, U.K.). Serial dilutions were made with sterile peptone from the different disinfection solutions or treatments, and also for the innoculum solution. For the initial loads of mesophiles on vegetables, samples were pour-plated into plate count agar (Difco, Becton, Dickinson and Co., Sparks, MD). Dishes were incubated at 35 °C for 48 h and then mesophiles were counted. For *E. coli* dilutions were pour-plated on EMB Agar (Neogen©, Lansing, MI). Dishes were incubated at 35 °C for 48 h and then bacteria were counted.

2.3. Disinfection treatments

2.3.1. Ultraviolet light

The ultraviolet treatment was applied in a cabinet equipped with a Sylvania germicidal lamp, G30 T8 RG3 30W Hg (Japan). The ultraviolet source has a maximum radiation peak at 253.7 nm. Experiments were conducted at room temperature (24 °C). Two working distances were used, 31 and 70 cm; vegetables were placed at these distances from the UV irradiated lamp. Samples were placed on sterile plastic petri dishes. Fluence was calculated as described by Bolton and Linden (2003) having two values, 1.6 mW/cm^2 and 0.65 mW/cm^2, for the shortest and longest distances, respectively. Processing times were 0, 3, 6, 9, 12, 15, 30 and 60 min. At the end of the longest exposure time, temperature was 25 (±0.5) °C. After treatment, samples were aseptically collected in sterile plastic bags with 0.1% peptone solution and processed for microbiological counts.

2.3.2. Ozone

Ozone was generated with an air cooled corona discharge equipment model lab 11 (Pacific Ozone, Egret Court Benicia, CA) connected to a packed bed reactor (7.3 cm internal diameter) with an approximately 0.1 cm bed depth. The batch reactor is made of 316 stainless steel (SS) and the connectors are made of 304 SS, 316 SS or PFTE. A thermocouple type K (Omega Engineering, Inc. Stamford, CT, USA) was installed inside the chamber to record the temperature profile during experiments to ensure a nonthermal treatment. A concentration of 5 ppm of ozone was used for the experiments, using a voltage of 3.4 V, flow rate of 2 standard lpm and pressure of 6 psi. Samples were placed on the bed during 3, 6, 9, 12 and 15 min. At the exit, the ozone concentration of the chamber was confirmed with a precision gas mass flow meter (Apex, Canton, GA). Equipment was calibrated with an iodine wet chemistry method to quantify ozone concentration (Rakness, Henry, & Langlais, 2000).

2.3.3. Chlorine solutions

Three different concentrations of sodium hypochlorite were prepared from a solution (6% v/v) of ultra germicidal bleach (Food Services America, Inc., Seattle, WA). Sterile water was mixed with sodium hypochlorite to have a final concentration of 50, 100 and 200 ppm (500 ml each). Chlorine concentration was verified with LaMotte® chlorine test papers (Chestertown, MD). The pH of the chlorine solution was adjusted to 6.5 in accordance with CFSAN