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Low dose UV-C illumination as an eco-innovative disinfection system on minimally processed apples

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ABSTRACT

In this study, the efficacy of UV-C illumination for inactivate *Escherichia coli*, *Listeria innocua* or *Salmonella enterica*, individually or in a mixture, *in vitro* and on apple slices was determined. Apple slices inoculated with a 10^7 cfu/mL suspension of above indicated pathogens were irradiated on both sides with UV-C illumination, with doses of 0.5 and 1.0 kJ/m^2. UV-C illumination disinfection efficacy was compared to that of washings with sodium hypochlorite at 100 ppm of free chlorine and with distilled water. Bactericidal activity of each treatment was assessed after 30 min and after 7 and 15 days of storage at 4 °C. Results showed that UV-C illumination at 1.0 kJ/m^2 could be an alternative to the wash with hypochlorite solutions. On the *in vitro* study, these doses completely inhibited the growth of the three bacteria either as pure cultures or in a mixture. In fresh-cut apple, the pathogens were also affected by the UV-C illumination, the 1.0 kJ/m^2 dosage being the one that resulted in higher bacteria inhibition in almost every case. The UV-C treatment did not affect the quality properties of fresh-cut apple.

1. Introduction

Consumption of fresh produce has increased over recent years, due to a tendency of adopting healthier food habits, and has led to the appearance of minimally processed (fresh-cut) products that are ready-to-eat. However, this growing demand raises the need for increase shelf-life and safety of these products. Fresh produce can be a vehicle for the transmission of foodborne pathogens, since they can be easily contaminated with microorganisms during production and processing. Contamination levels after harvesting can range from 3 to 7 log units, depending on the season and type of product (Ölmez and Kretzschmar, 2009).

In recent years, the number of outbreaks of human infections associated with the consumption of minimally processed products and unpasteurized fruit juices has increased. The major concerns are with enteric pathogens such as *Escherichia coli* O157:H7 and *Salmonella* spp. that have fast growth rates and low infectious doses (Martin, 2007). Salmonellosis and *E. coli* O157:H7 infection have been linked to watermelon, tomatoes, seed sprouts, melons, apple or orange juice (Blostein, 1993; del Rosario and Beuchat, 1995; Beuchat, 1996; Butler, 2000; Krause et al., 2001; Greene et al., 2008; Munnoch et al., 2009; Muranyi, 2012). Furthermore, listeriosis remains a great public health concern, as it has one of the highest case fatality rates of all the foodborne infections in Europe (20–30%) (Martin, 2007). The increase in reported outbreaks related to fresh fruit and vegetables may be the result of several causes. The per capita consumption of fresh produce has increased in developed countries. The demand for fresh produce year-round implies an increase in imports of these products from countries with different hygienic and sanitary conditions as well as the introduction of other pathogens (Lynch et al., 2009). Changes in processing, with more in-field cutting, coring and packaging, changes in the distribution systems, pathogens with different level of virulence, immunological changes among population segments and global trade, could be other reasons for the increase in outbreaks.

Fresh-cut fruit are more susceptible to foodborne pathogens because their natural barriers are removed. Several studies have demonstrated that *E. coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* could survive and/or grow in a range of minimally processed fruit such as apples (Dingman, 2000; Alegre et al., 2010a), honeydew melon (Leverentz et al., 2001, 2003), peaches (Alegre et al., 2010b), melon and pineapple (Abadias et al., 2012) and oranges (Lourenço et al., 2012) at temperature of 10 °C or higher.

Washing fresh-cut products with sanitizing solutions is the only step in which a reduction of microbial contamination can be achieved (Allende et al., 2009; Ölmez and Kretzschmar, 2009). Chlorine (sodium hypochlorite solution, 50–200 mg/L for 1–2 min) is the most common sanitizer used in fresh-cut industry (Beuchat, 1998).

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However, chlorination is related to environmental and human health risks. Chemical reactions of chlorine with organic matter may create toxic compounds, such as trihalomethanes (Allende et al., 2009; Gil et al., 2009). Adding to this, the scarcity of water resources is another environmental problem that has to be considered. Among the different industries, the food industry ranks third in water consumption and wastewater discharge rates, after the chemical and refinery industries (Casani et al., 2005). Thus there is a need to reduce or replace the use of chlorine as a disinfectant, particularly in the fresh-cut industry.

Ultraviolet light illumination is a non-thermal disinfection technology that can be used in fresh-cut industry. Is easy to use, is lethal to most type of microorganisms (Bintsis et al., 2000), does not generate chemical residues (Guerreo-Beltrán and Barbosa-Cánovas, 2004) and is a dry cold process that can be effective at low cost (Bachmann, 1975). UV-C light has maximal microbiocidal effect at 254 nm. UV-C doses ranging from 0.5 to 20 kJ/m^2 inhibit microbial growth by inducing the formation of pyrimidine dimers which distort the DNA helix and block microbial cell replication (Escalona et al., 2010). Cells become unable to repair their damaged DNA and die. The effectiveness of UV-C seems to be temperature independent (in the range from 5 to 37 °C), but depends on the illumination incidence, determined by the structure and topography of the surface of treated produce (Bintsis et al., 2000; Gardner and Shama, 2000), the fluence (J m^{-2}) and the relative position of the source and the sample.

The purpose of this work was to evaluate the effect of different doses of UV-C illumination on reducing the populations of *E. coli* O157:H7, *Listeria innocua* and *Salmonella enterica* subsp. *enterica* inoculated individually and in a mixture in *in vitro* and in fresh-cut apples.

2. Materials and methods

2.1. Bacterial strains

Non-toxicogenic strains of *E. coli* O157:H7 NCTC 12900, *L. innocua* CECT-910 and *S. enterica* subsp. *enterica* Michigan ATCC BAA-709 were used in this study. *L. innocua* has been used as a model organism for *L. monocytogenes* (Francis and O'Beirne, 1997). The bacterial strains were maintained on Tryptone Soy Agar medium (TSA, Oxoid) at 4 ± 1 °C. Prior to the experiments each microorganism was sub-cultured for 24 ± 2 h at 37 ± 1 °C on TSA and then in 50 mL of Tryptone Soy Broth medium (TSB, Oxoid) and incubated at 150 rpm for 24 ± 2 h at 37 ± 1 °C. To recover cells, each bacterium was centrifuged at 8000 rpm for 15 min and the pellet was resuspended in 50 mL of saline peptone [(8.5 g/L NaCl (Panreac) and 1 g/L peptone (Panreac)]. Inoculums with the appropriate concentration were prepared by adjusting the suspension according to a standard curve with a spectrophotometer (Spectrophotometer UV-Vis, 175 Shimadzu-UV160, USA) measuring the transmittance at 420 nm. Concentrations applied were confirmed using the Miles and Misra surface colony count method (1938), drops of 20 μL of 10-fold dilutions were placed in triplicate onto the surface of the TSA medium. Drops were left to be fully absorbed before inverting and incubating the plates at 37 ± 1 °C for 24 ± 2 h. Colonies were counted and by pathogen the results were expressed as a reduction of cfu/mL when compared with the population in control plates.

2.2. UV-C illumination conditions

The UV-C equipment consisted in a cabinet of 100 cm × 100 cm × 50 cm with two sets of 6 unfiltered germicidal lamps (Philips, TUV 25W G25 T8 Longlife). One set was suspended on the top and the other one was placed on the bottom of the UV-C chamber. The fresh-cut apples were placed between the UV-C lamps over a net. In order to determine the UV-C illumination intensity of the lamps, a radiometer (UVX Radiometer, UVP Inc., USA) was placed at the same distance as the commodities (15 cm). The applied UV-C intensity was calculated as a mean of 20 readings in different places taken at each side of the net. Light intensity was kept constant, and the applied doses varied by modifying the exposure time. Prior to use the UV-C lamps were allowed to stabilize by turning on 10 min before treatment. A ventilation device was installed in the back of the box to avoid temperature increase because of UV-C illumination.

2.3. In vitro antimicrobial activity of UV-C illumination against E. coli, L. innocua, S. enterica or a mixture of the three microorganisms

A 10^8 cfu/mL pure culture suspension of *E. coli*, *L. innocua*, *S. enterica* or a cocktail of the three microorganisms was prepared and series of 10-fold dilution were made in SP. The concentrated and the diluted cultures were plated in Petri dishes (three drops of 10 μL) onto a specific medium for each pathogen: Sorbitol MacConkey Agar (Biokar) for *E. coli*, and Hektoen Agar (Biokar) for *S. enterica*, and Palcam Agar (Biokar) for *L. innocua*.

Three sets of dishes were made. One set was submitted to UV-C illumination immediately after plate inoculation and the other was treated 4 h after inoculation. The UV-C doses applied were 0.5 and 1.0 kJ/m^2. Untreated but inoculated plates consisted in the third set and were used as control. Petri dishes were incubated at 37 ± 1 °C for 48 ± 2 h. Colonies were counted and the results expressed as the reduction of cfu/mL of each pathogen when compared to control plates. Control plates were counted only in diluted culture.

The experiment was conducted three times.

2.4. Fresh-cut apple preparation

'Golden Delicious' apples used in this study were purchased in a packinghouse and stored at 0.5 ± 0.5 °C and 90% RH before processing. Apples were washed, sanitized by immersion, rubbed in a sodium hypochlorite solution (Panreac) at 0.5% for 30 s, and let to dry. Apples were then aseptically cut using a cutting instrument, suitable for apples, in pieces of 10 g each, without core tissue, and with skin on.

2.5. Antimicrobial activity of UV-C illumination on fresh-cut apples inoculated with pure culture of E. coli, L. innocua or S. enterica

Apple pieces were submerged into a 500 mL of *E. coli*, *L. innocua* or *S. enterica* pure culture suspension at 10^7 cfu/mL for 3 min with 150 rpm orbital agitation. Inoculated samples were air-dried in a laminar flowhood for 30 min before receiving the treatment. For each inoculated microorganism, apple pieces were divided into 5 batches of 12 pieces each. Two of the batches were treated for 5 min at 150 rpm orbital agitation in flasks containing 500 mL of distilled water (DW) or sodium hypochlorite solution (SH) at 100 mg/L of free chlorine (pH 6.5), determined by using a free and total chlorine photometer (model HI9133, HANNA Instruments, Woonsocket, RI, USA). After SH treatment, apple pieces were drained and rinsed with cold distilled water for 3 min at 150 rpm orbital agitation. These two batches were left to dry in a laminar flowhood for 30 min.

The other batches were treated with UV-C illumination at doses of 0.5 and 1.0 kJ/m^2 each. Untreated but inoculated pieces were used as control.

Apple pieces from each treatment were divided into 3 batches (4 pieces each) and packed in oriented polystyrene lidded trays