Efficacy of Electrolyzed Water to Inactivate Foodborne Pathogens on Fresh-Cut Apples

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Abstract

Chlorine is the most common disinfectant used in the fresh-cut industry but nowadays environmental and health risks have led to the need to find new sanitizers. Electrolyzed water (EW) appears to be a promising alternative. In this work, disinfection efficacy of acidic (AEW) and neutral (NEW) electrolyzed water in fresh-cut apple slices inoculated with *Escherichia coli*, *Listeria innocua* or *Salmonella choleraesuis* was studied. Apple slices were inoculated with a 10^7 cfu/ml suspension of pathogens and treated with the sanitizer EW solutions, with 100 or 50 ppm of free chlorine solutions and with distilled water. Population reduction was determined 30 min after washings and untreated apple slices were used as control in all assays. AEW100 was the treatment with more effective bactericidal activity followed by NEW100 and AEW50. EW had higher or similar efficacy than chlorine treatments in all tested conditions.

INTRODUCTION

Fresh-cut produce is a rapidly rising sector of the horticultural industry because minimally processed fruits and vegetables offer many advantages to the final consumer. Fresh fruits and vegetables may contain a high contamination level after harvest, ranging between 3 and 7 log colony units depending on the season and type of produce (Ölmez and Krestzschmar, 2009). Therefore, fresh produce can be a vehicle for the transmission of bacterial, parasitic and viral pathogens that can cause human illnesses. Fresh fruit products have been associated with outbreaks of illness caused by *E. coli* O157:H7 and *Salmonella* spp. (del Rosario and Beuchat, 1995; Beuchat, 1996; Burnett and Beuchat, 2000). Moreover, several studies have shown that *E. coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* can grow in fresh fruit tissues, such as in apples, (Beuchat, 1996; Janisiewicz et al., 1999; Leverentz et al., 2001; Park et al., 2001; Alegre et al., 2010; Oliveira et al., 2010).

Washing fresh-cut fruits with sanitizing solutions is the only step in the process where a reduction of microbial contamination can be obtained (Allende et al., 2009; Ölmez and Kretzschmar, 2009). Chlorine applied at concentrations ranging from 50 to 200 ppm with a contact time of 1-2 min (Beuchat, 1998) is the most common sanitizing procedure used in the fresh-cut industry. However, there are some concerns about its efficacy as well as the health risks involved, since it has been proven that these risks are associated with the formation of toxic compounds, such as trihalomethanes (Allende et al., 2009; Gil et al., 2009). As a result, there is a trend to eliminate or reduce chlorine as a disinfectant in the fresh-cut industry.

Some products have been tested as alternative disinfectants such as ozone (Selma et al., 2008; Ölmez and Akbas, 2009), UV-C (Allende and Artés, 2003; Gómez et al., 2010), organic acids (Akbas and Ölmez, 2007), chlorine dioxide (Rodgers et al., 2004) or peroxyacetic acid (Baert et al., 2009).

In recent years electrolyzed water (EW) has been considered a new eco-innovative technique showing good results as a sanitizer (Wang et al., 2006; Abadias et al., 2008). There are two types of EW with sanitizer properties, acidic electrolyzed water (AEW) and neutral electrolyzed water (NEW). These solutions are generated by electrolysis of a diluted NaCl solution (0.5-1.0%). AEW has a strong bactericidal effect on most known pathogenic bacteria due to its low pH (2-4) and high oxidation-reduction potential (ORP >1000 mV). In addition, because it contains active oxidizers like hypochlorous acid (Kim et al., 2000; Len et al., 2000), it is effective in killing food-borne pathogens under in vitro conditions and in reducing microbial counts and pathogens on vegetables. NEW has also a strong bactericidal effect when the pH is between 5.0-8.5 and the ORP is between 500-700 mV.

Several studies have revealed that EW is effective in reducing or eliminating pathogenic microorganisms on minimally-processed vegetables (Yang et al., 2003; Gomez-Lopez et al., 2007; Abadias et al., 2008; Koide et al., 2009). However, its efficacy on fresh-cut fruits has only been reported in the reduction of *E. coli* in fresh-cut apples (Wang et al., 2006, 2007; Nunes et al., 2010).

The aim of this work was to evaluate the efficacy of AEW and NEW on the reduction of the population of *Escherichia coli* O157:H7, *Listeria innocua* and *Salmonella choleraesuis* subsp. *choleraesuis* on fresh-cut apples.

MATERIAL AND METHODS

Bacterial Cultures

A non-toxicogenic strain of *E. coli* O157:H7 NCTC 12900, *Listeria innocua* CECT-910 and *Salmonella choleraesuis* subsp. *choleraesuis* (Smith) Weldin serotype Michigan, ATCC BAA-709 were used in this study. *Listeria innocua* has been used as a model organism for *L. monocytogenes* (Omary et al., 1993; Francis and O'Beirne, 1997). The bacterial strains were maintained on solid TSA (Tryptone Soy Agar) medium at 4±1°C. Prior to experiments each microorganism was sub-cultured for 24±2 h at 37±1°C on TSA and then in 50 ml of TSB (Tryptic Soy Broth) medium and incubated at 37°C and 150 rpm for 24±2 h. 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 and 1 g/L peptone). Inoculums with 10^7 cfu/ml were prepared by adjusting the suspension concentration according to an optical density standard curve (420 nm). Concentration of cells were confirmed by plating drops of 20 µl in triplicate onto the surface of the TSA medium using Miles and Misra method (1938) and incubated at 37±1°C for 24±2 h.

Apple Slice Preparation

'Royal Gala' apples used in this study were purchased in a local supermarket and stored at 0.5±0.5°C before processing. Apples were washed, then sanitized by immersion and rubbed in a sodium hypochlorite solution (commercial bleach) at 0.5% for 30 s, and dried. After drying, they were aseptically cut in slices of 25 g each, with a sterilized stainless-steel knife. The core was removed and only the portions with the skin were used.

Effect of Inoculation Time

To determine the optimal conditions to inoculate the pathogens, a study of inoculation and drying times was made in apple plugs of 1 g (1 cm long and radius of 0.5 cm taken with a sterile cork borer). Disinfected apples were prepared, as previously described and inoculated during 1, 2 or 3 min with drying times of 0.5, 1, 2, 3 or 4 h (data not shown). As a result, the time of 3 min of agitation and 30 min of drying was chosen for the assays.

Preparation of Treatment Solutions

AEW and NEW were generated using an EW generator (Envirolyte EL-400, Envirolyte Industries International Ltd., Estonia) following manufacturer instructions. A saturated sodium chloride solution was pumped into the equipment and the current passing through the EW generator was set at 20-23 A. AEW and NEW were collected from each outlet in flasks and stored at 4°C. Solutions of AEW and NEW at 50 and 100 ppm of free chlorine were also prepared by diluting in distilled water immediately before treatment. Distilled water (DW) and sodium hypochlorite (H) solution at 50 and 100 ppm of free chlorine were used as control. Sodium hypochlorite solutions were prepared by diluting a 4% sodium hypochlorite solution (commercial bleach) with DW. All solutions were stored at 4°C and used within 1 h. The properties of each solution were determined, including ORP, pH and free chlorine concentrations. All measurements were made immediately before treatments at 4°C. The ORP and pH were measured with the use of a pH-meter (model GLP-21, Crison, Barcelona) using an ORP electrode (ref. 5261) and a pH electrode (ref. 5202) respectively. Free chlorine concentrations were determined by using a free and total chlorine photometer (model HI9133, HANNA Instruments, Woonsocket, RI, USA).

Antimicrobial Activity of AEW and NEW on Fresh-Cut Apples Inoculated with *E. coli*, *L. innocua* or *S. choleraesuis*

Apple slices were submerged in a 10^7 cfu/ml suspension of *E. coli*, *L. innocua* or *S. choleraesuis* for 3 min with 150 rpm orbital agitation. Inoculated samples were air-dried under a laminar flow hood for 30 min before receiving the washing treatment. Inoculated apple slices were divided in 8 batches of 10 slices each. The time of exposure to the washing treatments was selected based on the studies carried out in our laboratory. Previously, we determined the effect of treatment exposure time (3 and 5 min) using *E. coli*, and the results obtained showed that higher reductions were achieved with 5 min exposure time (Graça et al., 2010). Each batch was treated for 5 min at 150 rpm agitation in flasks containing 500 ml solution of one of the following treatment solutions: DW, H100, H50, AEW100, AEW50, NEW100 and NEW50. After treatment, apple slices were drained and rinsed twice with cold distilled water for 3 min at 150 rpm and left to dry under a laminar flow hood for 30 min. Untreated, but inoculated, samples were used as control and each treatment was done in triplicate. Once dried, apple slices from each treatment were divided in 2 batches (5 slices each). One was used immediately and fruits from the other batch were packed in polyethylene bags (0.065 mm thick) and kept at 4±0.5°C for 5 days. Concentration of each bacterium on apple samples was determined after drying and 5 days after cold storage. For each disinfection treatment, 25 g of apple slices were transferred into sterile Stomacher bags and mixed with 225 ml of sterile saline peptone and homogenized for 2 min in a Stomacher as described before. Homogenates were serially diluted in saline peptone and drops of 20 µl in triplicate were plated onto the surface of the TSA medium using Miles and Misra method (1938) and incubated at 37±1°C for 24 h. Colonies were counted and the results expressed as cfu g^{-1} of apples. This experiment was repeated three times.

Statistical Analysis

Values represent the means of 3 different experiments, with 3 replicates per treatment per experiment. Data were subjected to analysis of variance and Duncan's multiple range tests using SPSS v.16.0 software (SPSS Inc., USA). Significant differences in survival population values were established by the least significant difference at the 0.05 level of significance.

RESULTS AND DISCUSSION

Inoculation and dry time did not influence the initial population of *E. coli* O157:H7 on apple slices (data not shown), and the time of 3 min of agitation and 30 min of drying was chosen for the assays. This procedure will assure an adequate and

homogeneous contact between pathogen and fruit samples, and simulate the period between fresh-cut fruit contamination and the disinfection treatment, in case that microbial contamination occurs at some point during peeling, cutting or slicing, and sanitizing process in commercial conditions. The initial viable cells recovered after 30 min of inoculation for 3 min on unwashed apple slices were 6.35, 5.30 and 5.73 log cfu/g for *E. coli*, *L. innocua* and *S. choleraesuis*, respectively.

In fact it is necessary to establish a standardized method to determine the efficacy of sanitizers, including the selection of strains, preparation of inoculums, inoculation procedure, detection and enumeration recovery of pathogens or groups of microorganisms from fresh-cut fruit before designing experiments to calculate the efficacy of treatments with disinfectants (FDA, 2001).

Electrolyzed water and sodium hypochlorite treatments were shown to be effective for reducing *E. coli*, *L. innocua* and *S. choleraesuis*. The bactericidal activity of the treatments depended upon the type of sanitizer, within the same sanitizer upon the concentration of free chlorine and pathogen (Table 1).

For *E. coli* the higher bactericidal activity was observed in apple slices washed with AEW100 and AEW50, obtained reduction values of 2.47 and 2.09 log cfu/g, respectively. For concentrations of 100 ppm of free chlorine, reductions observed with NEW and H were less than 2 log units and no differences were observed between these two treatments. The lowest bactericidal activity was obtained with NEW50, and no differences between populations on apple slices treated with NEW50 and DW were observed.

Treatments with AEW, NEW and H significantly decreased the viable cells of *L. innocua* in apple slices. The population was reduced more than 1.20 log cfu/g with AEW100 and with H100. Washing with AEW50 resulted in a reduction of 1.15 cfu/g. The inactivation of *L. innocua* achieved with the other treatments was similar and lower than 1.00 log cfu/g. DW did not reduce *L. innocua* population. In general, the weakest bactericidal effect was observed against *L. innocua*, maybe because this species is less sensitive to chlorine than the other foodborne pathogens. In fact, Nguyen-the and Carlin (1994) suggest that inactivation of *L. monocytogenes* on vegetables by chlorine is limited.

The population of *S. choleraesuis* was reduced more than 1.40 log cfu/g with AEW100 and AEW50 washing treatments. Both NEW washings reduced more than 1.2 log cfu/g. H100 was able to reduce *S. choleraesuis* population in 1.09 log cfu/g, followed by H50 with 0.83 log cfu/g of reduction. DW was the treatment with the lowest reduction, 0.65 log cfu/g.

Results obtained in this study demonstrated that, in general, AEW had a stronger antimicrobial effect than NEW and H. AEW has higher ORP and lower pH. The high ORP could cause modification on metabolic fluxes and ATP production and low pH may affect the outer membrane of bacterial cells and facilitate the entry of hypochlorous acid (HOCl) into microbes (McPherson, 1993). Hypochlorous acid is the main active agent of AEW and NEW (Len et al., 2000) and is the form of free available chlorine that has the highest bactericidal activity against a broad range of microorganisms. Hypochlorous acid produces hydroxyl radical (OH) that acts on microorganisms. Therefore the higher concentration of HOCl present in AEW produces more OH which combined with higher ORP and lower pH results in more effective antimicrobial activity.

The advantages of using electrolyzed water in the fresh cut industry to disinfect fruit are a lower adverse impact on the environment and human health, since no hazard chemicals are added during processing (Huang et al., 2008). In addition it is less expensive than other sanitizing techniques, once the initial investment is made to purchase the equipment, the only expenses are water, sodium chloride and electricity (Walker et al., 2005).

CONCLUSIONS

In this study washings with electrolyzed water (EW), either acidic (AEW) or neutral (NEW), show significant reduction of populations of *E. coli*, *L. innocua* and

S. choleraesuis on fresh-cut apple slices. AEW with 50 ppm and NEW with 100 ppm of free chlorine had more or comparable efficacy than chlorinated water at 100 ppm of free chlorine. In some cases, AEW50 efficacy was similar or superior to that of NEW100 or H100. It would be interesting, to test the effect of EW on various combinations of foodborne pathogens, since, as suggested in some works, population size does not affect the effectiveness of treatments as well as the effect of cold storage in inoculated and disinfected fresh-cut fruits.

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Tables

Table 1. Survival population (log cfu/g) of *Escherichia coli*, *Listeria innocua* and *Salmonella choleraesuis* population on inoculated fresh-cut apples washed for 5 min with different solutions, followed by two rinses for 3 min with distilled water. Fruit slices of 25 g each were inoculated by dipping in 10^7 cfu/ml suspension of each foodborne pathogen.

	AEW[a]		NEW[b]		H[c]		DW[d]	Untreated fresh-cut apples
	100 ppm[e]	50 ppm	100 ppm	50 ppm	100 ppm	50 ppm	0 ppm	
E. coli	3.88 e	4.26 d	4.37 cd	4.90 b	4.46 cd	4.60 c	4.62 c	6.35 a
L. innocua	4.08 c	4.15 bc	4.39 b	4.42 b	4.09 c	4.41 b	5.30 a	5.30 a
S. choleraesuis	4.32 e	4.22 e	4.51 de	4.48 de	4.64 cd	4.90 c	5.08 b	5.73 a

[a] acidic electrolyzed water.
[b] neutral electrolyzed water.
[c] sodium hypochlorite.
[d] distilled water.
[e] ppm of free chlorine.
Rows with different letters indicate significant difference between treatments using LSD ($P<0.05\%$). Values are the mean of 3 experiments with 3 replicates each and bars indicate standard deviation of means.