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Highlights

The growth of *E. coli*, *S. enterica*, and *Listeria* spp. on fresh-cut pear was studied

UV-C efficacy on the inactivation of the bacteria on fresh-cut pear was assessed

The effect of electrolyzed water on foodborne bacteria population was measured

Fresh-cut pear is a good substrate for the survival and growth of foodborne bacteria

UV-C was more effective than electrolyzed water to reduce foodborne bacteria on pear

Growth of *Escherichia coli*, *Salmonella enterica* and *Listeria* spp., and their inactivation using ultraviolet energy and electrolyzed water, on 'Rocha' fresh-cut pears

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ABSTRACT

The present study aimed at evaluating the growth of *Escherichia coli*, *Salmonella enterica*, and *Listeria* spp. and studying the efficacy of Ultraviolet-C (UV-C) irradiation, acidic electrolyzed (AEW) and neutral electrolyzed (NEW) waters in the reduction of these bacteria on 'Rocha' pear. Fresh-cut pieces were inoculated and incubated at 4-20 °C for 8 days. Inoculated pears were treated with UV-C (2.5-10 kJ/m^2), AEW, NEW and sodium hypochlorite (SH) and microbiological and quality parameters were evaluated. The three bacteria, inoculated at 6.1-6.2 log cfu/g, grew on the pear at high growth rates at 12 and 20 °C reaching populations of 8.1-8.6 log cfu/g, in 24 h. At 8 °C the microorganisms increased their populations by at least 1 log cfu/g in three days. At 4 °C adaptation phases of less than 24 h for *Listeria* spp. were measured before exponential growth occurred and the enterobacteria did not grow despite having survived for 8 days. AEW and NEW caused microbial reductions similar to SH, of approximately 1 log cfu/g, while the best UV-C dose (7.5 kJ/m^2) of at least 2.4 log cfu/g. Fresh-cut pears were a good substrate for foodborne bacteria emphasizing the importance of preventing contaminations and cross contaminations. The UV-C was more effective than the chemical decontaminations, as it provided superior microbial reductions without greatly affecting the quality of pears.

Keywords: 'Rocha' fresh-cut pears, *Escherichia coli*, *Salmonella enterica*, *Listeria* spp., Ultraviolet-C, Electrolyzed water

1. Introduction

The safety and the increase of shelf-life of minimally processed foods are two major challenges for the industry as fresh produce may contain high microbial levels after harvesting and can be easily contaminated with foodborne microorganisms during the processing (Graça, Santo, Esteves, Nunes, Abadias & Quintas, 2015, Graça, Esteves, Nunes, Abadias & Quintas, 2017; Ölmez and Kretzschmar, 2009; Parish, Beuchat, Suslow, Harris, Garrett, Farber & Busta, 2003; Ramos, Miller, Brandão, Teixeira & Silva, 2013).

The natural microbiota of raw fruits and vegetables is usually nonpathogenic for humans and is present at the time of consumption. However, during primary production and processing, the food can be contaminated with pathogens from human, animal or environmental sources (Brandl, 2006). Fresh fruit products (apple juices, tomatoes, watermelon, mango, cantaloupe, berries) have been responsible for outbreaks caused by pathogenic bacteria such as *Escherichia coli* O157:H7, *Salmonella enterica* and *Listeria monocytogenes* (Ölmez and Kretzschmar, 2009; Parish et al., 2003; Ramos et al., 2013). The growth of pathogens on food during distribution/storage is thought to be determinant to most outbreaks (Codex Alimentarius Commission, 1999) and several studies have demonstrated the capacity of pathogenic bacteria to survive and/or grow at different temperatures in minimally processed fruits (Abadias, Alegre, Oliveira, Altisent & Viñas, 2012; Alegre, Abadias, Anguera, Oliveira & Viñas, 2010a; Alegre, Abadias, Anguera, Usall & Viñas, 2010b; Dingman, 2000; Lourenço, Graça, Salazar, Quintas & Nunes, 2012; Santo, Graça, Nunes & Quintas, 2016). Moreover, different produce differ in the ability to support the growth of bacteria as reported for *L. monocytogenes* (Hoelzer, Pouillot & Dennis, 2012). The processing operations inherent to the minimal processing which include cutting, dicing, washing, decontamination and packaging are determinant to the contamination levels and for the microbial growth behavior. Operations such as cutting and dicing increase the availability of nutrients and contribute to the dissemination of microorganisms and their growth. Additionally, the capacity of microorganisms to produce biofilms on fresh produce may enhance their survival and growth and enable the bacteria to persist and withstand washing and antimicrobial treatments. *Salmonella* Typhimurium embedded in a biofilm matrix resisted

sodium hypochlorite (NaOCl) at concentrations above 500 mg/L, while planktonic cells were sensitive to less than 50 mg/L (Scher, Romling & Yaron, 2005).

Sodium hypochlorite (50 to 200 mg/L, during 1-2 minutes) is the most widespread disinfectant applied in the fresh-cut industry, although it can cause problems to man and the environment due to the generation of potentially harmful by-products such as gases, trihalomethanes and chloramines. Additionally, its efficacy is dependent on pH, organic material and the physiologic state of microorganisms, and its use is prohibited in some European countries. As a consequence, alternative chemical and physical decontamination methods are studied (Beuchat, 1998; Ramos et al., 2013).

Short wave Ultraviolet-C (UV-C) radiation and electrolyzed water (EW) are two non-thermal decontamination technologies that have been tested as alternatives to chlorine. Different studies have reported that UV-C light at 254 nm in doses from 0.5 to 20 kJ/m^2 reduces the number of microorganisms, thus contributing to the extension of shelf-life while maintaining and/or improving the overall safety and quality of fresh-cut fruit (Bintsis, Litopoulou-Tzanetaki & Robinson, 2000). The main injuries of UV-C on microorganisms, especially on *E. coli*, result from membrane alterations on phospholipids, secondary structures of proteins, and polysaccharides and changes on structures of DNA/RNA (Syamaladevi, Sablani, Insan, Adhikari, Killinger, Rasco, Dhingra, et al. 2013). This technique was successfully applied to reduce microbial contamination and/or to extend shelf-life in mango and pineapple (George, Razali, Santhirasegaram & Somasundram, 2015), watermelon (Artés-Hernández, Robles, Gómez, Tomás-Callejas & Artés, 2010), kiwifruit (Beirão-da-Costa, Moura-Guedes, Ferreira-Pinto, Empis & Moldão-Martins, 2014), apples (Graça, Salazar, Quintas & Nunes, 2013), apricot (Yun, Yan Fan, Gurtler & Phillips, 2013) and melon (Manzocco, Da Pieve & Maifreni, 2011). Moreover, the UV-C irradiation has been associated to the enhancement of antioxidant activity measured in mango and pineapple (George et al., 2015) and in watermelon (Artés-Hernández et al., 2010), to the increase of peroxidase activity in cantaloupe (Lamikanra,

Kueneman, Ukuku & Bett-Garber, 2005), to the induction of the production of anthocyanins and stilbenoids (Ramos et al., 2013) and the promotion of enzymatic stability in fresh-cut fruit through the inactivation of pectate lyases (Manzocco, Dri & Quarta, 2009a) and polyphenoloxidases (Manzocco, Quarta & Dri, 2009b) in apples. The major advantages of UV-C irradiation reside in the fact that it is a dry cold process that does not require expensive or high energy consuming equipment, involve extensive safety equipment or leave toxic residues. Furthermore, it has broad-spectrum microbicidal activity and is relatively inexpensive (Artés, Gómez, Aguayo, Escalona & Artés-Hernández, 2009; Guerrero-Beltrán and Barbosa-Cánovas, 2004; Ramos et al., 2013). However, some disadvantages need to be mentioned, such as the possible induction of alterations that change the appearance of the samples (Rico, Martin-Diana, Barat & Barry-Ryan, 2007) and the lack of penetration capacity, causing only a superficial disinfection (Bintsis et al., 2000).

EW has been reported to have a great microbicidal activity against several pathogenic and spoilage microorganisms and has also the advantage of neutralizing harmful substances such as cyanides and ammonium (Huang, Hung, Hsu, Huang & Hwang, 2008; Ramos et al., 2013). It is produced through the electrolysis of a sodium chloride solution in electrolytic cells where two types of EW can be formed: acidic electrolyzed water (AEW), produced at the anode, and neutral electrolyzed water (NEW) produced at the cathode. AEW has low pH (2-4), high oxidation-reduction power (ORP) (> 1000 mV) and contains oxygen gas, chlorine gas, hypochlorite ion, hypochlorous acid and hydrochloric acid. NEW is characterized by pH values of 5 to 8.5 and ORP values of 500 to 700 mV and contains hydrogen gas and sodium hydroxide (Huang et al., 2008). Although the mode of action of EW is not clearly understood its antimicrobial activity may be related to the disruption it causes in the cell wall of bacteria (Osafune, Ehara & Ito, 2006) and to the high oxidizing potential of hypochlorous acid producing hydroxyl radicals ($^{\bullet}$OH) which act on cells and its components (proteins, nucleic acids) (Huang et al., 2008). Electrolyzed water has been used as a disinfectant for food processing equipment and has also been successfully applied to decontaminate fruits and vegetables, among other food. Its application contributes to the reduction of the microbial load

on blueberries (Kim and Hung, 2012), tomatoes and lettuce (Pangloli and Hung, 2011), broccoli (Martínez-Hernández, Navarro-Rico, Gómez, Otón, Artés & Artés-Hernández, 2015), lettuce, carrot and endive (Abadias, Usall, Oliveira, Alegre & Viñas, 2008) and cilantro (Wang, Feng & Luo, 2004). In fresh-cut apple, both AEW and NEW revealed microbiocidal activity on *E. coli*, *L. innocua* and *S. enterica* as described by Graça, Abadias, Salazar & Nunes, (2011). The main advantages of EW are its broad-spectrum microbicidal activity, its safety, as it is not corrosive to humans' health (skin, mucous membranes), is less reactive with organic material and has a less adverse impact on the environment (Huang et al., 2008). Nevertheless, the main limitations of this type of disinfection is that the solutions rapidly lose antimicrobial activity and may be involved in metal corrosion and degradation of synthetic resins, depending on the pH and free chlorine content, as referred by Huang et al., (2008).

'Rocha' pear (*Pyrus communis* L. cv Rocha) is a Portuguese variety being recognized as a Protected Denomination Origin (PDO) fruit. Its production reached 195,000 tons in 2013, accounting for 95 % of the national pear production from which about 30 % was exported. Due to its characteristics, namely flavor and texture, recently it began to be marketed as minimally processed fruit in restaurants, supermarkets and on airline travel caterings. Since no information is available on the capacity of foodborne pathogens to grow on 'Rocha' pear tissues and on the effect of decontamination technologies on fresh-cut pieces of this fruit, the aim of the present work was to study the growth of *E. coli*, *S. enterica* and *Listeria* spp. on minimally processed 'Rocha' pear at different temperatures and evaluate the efficacy of UV-C irradiation, acidic and neutral electrolyzed water on reducing the mentioned bacteria population, inoculated individually and in a mixture, in fresh-cut pears.

2. Methods

2.1. Pear preparation

The 'Rocha' (cv) pears used in the present study were purchased in an orchard and stored at 0.5±0.5 °C before processing. Pears were washed in running tap water and surface disinfected by dipping and scrubbing in a sodium hypochlorite solution (0.5 %) during 30 s. After drying at room temperature, pears were aseptically cut in pieces of 1 g each (1 cm long and radius 0.6 cm obtained with a sterile cork borer), without core tissue and skin. Pieces of 10 g each without core tissue and with the skin were prepared, using a cutting instrument, to perform the decontaminations and evaluate the quality of the fruit.

2.2. Microorganisms and preparation of inocula

The bacterial species used in the present work were *Escherichia coli* (the non-toxicogenic strain of *E. coli* O157:H7 NCTC 12900, *E. coli* ATCC 25922 and *E. coli* ATCC 10536), *Listeria innocua* CECT-910, *L. monocytogenes* C897 (Faleiro et al., 2003) and *Salmonella enterica* (subsp. *enterica* Michigan ATCC BAA-709 and *S.* Typhimurium ATCC 14029). The bacteria were stored at -80 °C and maintained on Tryptone Soy Agar (TSA) (Oxoid, Hampshire, UK) at 4±1 °C. Bacterial inocula used to contaminate the fruit, were cultivated on TSA and incubated during 24±2 h at 37±1 °C. Then, they were sub-cultured in 50 mL of Tryptic Soy Broth (TSB) (Biokar Diagnostics, Allonne, France) following an orbital incubation (VWR, Incubating Mini Shaker, USA) at 150 rpm at 37±1 °C. After 24 h, the bacterial cells were recovered by centrifugation at 9016 g for 15 min (Heraeus, Multifuge 1 L-R, Germany) and the pellet was resuspended in 50 mL of sterile saline peptone [8.5 g/L NaCl (Panreac, Barcelona, Spain) and 1 g/L peptone (Biokar)]. These suspensions were used as inocula of fresh-cut pear, after an adjustment of its concentration to 10^7 cfu/mL according to a standard curve, measuring the transmittance at 420 nm in a spectrophotometer (Spectrophotometer UV–Vis, 175 Shimadzu-UV160, USA). The concentrations of bacterial suspensions used as inocula were confirmed using the Miles and Misra (1938) surface colony count method. Drops of 20 µL of ten-fold dilutions were released in triplicate onto the surface of the TSA medium and plates were incubated at 37±1 °C for 24±2 h.

190 *2.3. Growth of E. coli, S. enterica and Listeria spp. on fresh-cut pears at different temperatures*

191

192 The growth of *E. coli, S. enterica* and *Listeria* spp. on fresh-cut pears at different temperatures
193 was performed on 1 g pear pieces previously prepared as described above. Pear portions were
194 submerged in 10^7 cfu/mL suspensions of *E. coli, S. enterica* and *Listeria* spp. separately, during
195 3 min at 150 rpm in an orbital shaker. After drying in a laminar flow hood (Bioquell,
196 Microflow, UK) during 30 min, samples were divided in 6 sets. Each set was divided in 4 other
197 groups each containing 4 pear pieces. One set was analyzed straightaway (Day 0). The other 5
198 sets were packed in biaxially-oriented polypropylene (BOPP) (0.030 mm thick) bags and each
199 one was stored at four different temperatures: 4±0.5 °C, 8±0.5 °C, 12±0.5 °C and 20±0.5 °C. At
200 each temperature, the population of the three different bacteria was enumerated, individually, on
201 the fresh-cut pear samples on days 1, 2, 3, 6 and 8, after the inoculation. The inoculated pear
202 portions (1 g) were transferred into sterile Stomacher bags, mixed with 9 mL of sterile saline
203 peptone and homogenized in a Stomacher (Model 400 Circulator, Seward, Norfolk, England)
204 during 2 min. Homogenates were serially diluted in saline peptone and aliquots of 20 µL were
205 plated in triplicate on the surface of Sorbitol MacConkey agar (Biokar Diagnostics) to count the
206 number of *E. coli*, on Palcam agar (Biokar Diagnostics) to evaluate the population of *Listeria*
207 spp. and on Hektoen agar (Biokar Diagnostics) to enumerate *S. enterica*. The evaluation of the
208 microbial populations was performed with the Miles and Misra method (Miles and Misra,
209 1938). Plates were incubated at 37±1 °C for 24±2 h (*E. coli* and *S. enterica*) or for 48±2 h
210 (*Listeria* spp.). Colonies were counted and the results expressed as colony forming units (cfu)
211 per gram of pears. In each sampling point, four replications were performed and the experiments
212 were repeated twice. The specific growth rates (day^{-1}), adaptation phases (Lag) (day) and final
213 microbial population (Final value) (log cfu/g) were calculated using the DMFit modeling tool
214 (http://modelling.combase.cc) (Baranyi and Roberts, 1994).

215

216 *2.4. UV-C treatment*

The UV-C treatments were performed in a chamber (100 cm x 100 cm x 50 cm) equipped with two sets of five unfiltered germicidal emitting lamps (Philips, TUV 25W G25 T8 Longlife). One set of lamps was placed horizontally on the top and the other one on the bottom of the radiation cabinet. The fresh-cut pears were placed on a net positioned midway between the UV-C lamps. The walls of the cabinet enhanced a homogeneous dispersion of the emitted light to allow irradiation of almost the whole food surfaces. The UV-C radiation intensity of the lamps was measured with a radiometer (UVX Radiometer, UVP. Inc, USA) placed at the same distance as the commodities (15 cm) and calculated as a mean of 20 readings in different places taken at each side of the net. The intensity of light was kept constant and the applied doses varied by modifying the exposure time. The UV-C doses selected to use as decontamination treatments on fresh-cut pears were 2.5, 5, 7.5 and 10 kJ/m^2 and will be referred to as UV2.5, UV5, UV7.5 and UV10, respectively (in the figures, tables and text).

2.5. Electrolyzed water

Acidic electrolyzed water (AEW) and neutral electrolyzed water (NEW) were produced with an electrolyzed water (EW) generator (Envirolyte EL-400, Envirolyte Industries International Ltd., Estonia) when a saturated sodium chloride solution was pumped into the equipment with the current set at 20–23 A, according to the instructions of the manufacturer. AEW and NEW were collected in flasks and kept at 4 °C until use (no more than one day). Solutions of AEW and NEW were prepared at 100 mg/L of free chlorine by diluting with distilled water previous to its application on the fruit.

UV-C irradiation treatments and AEW and NEW washings were compared with distilled water (DW) and sodium hypochlorite (SH) solutions at 100 mg/L free chlorine. SH solutions were prepared by diluting a 4 % sodium hypochlorite solution (AppliChem, Darmstadt, Germany) with distilled water. All solutions were stored at 4 °C and used within 1 h. The properties of each solution such as ORP, pH and free chlorine concentration were measured. ORP and pH

were measured with a pH-meter (Model GLP-21, Crison, Spain), using an ORP electrode (Crison 52-61) and a pH electrode (Crison 52-02), respectively. Free chlorine concentrations were determined using a free and total chlorine photometer (HANNA Instruments, model HI9133, Woonsocket, RI, USA). The AEW used in the decontamination treatments had a pH of 2.90 (±0.03), a ORP of 1121 (±3) mV and a free chlorine of 99 (±2) mg/L. The NEW applied in the fresh-cut pear was characterized by a pH of 8.20±0.11, a ORP of 754±5 mV and contained 102±2 mg/L of free chlorine.

2.6. Inactivation of E. coli, S. enterica and Listeria spp. (individually and in a mixture) on fresh-cut pears using UV-C irradiation and AEW and NEW

Fresh-cut pear pieces were immersed in a 10^7 cfu/mL suspension of *E. coli*, *S. enterica*, *Listeria* spp. individually, during 3 min with 150 rpm orbital agitation. The inoculation level was higher than expected through cross contamination to facilitate the enumeration of the bacterial reductions. Inoculated samples were air-dried in a laminar flow hood during 30 min before the application of the treatments.

Inoculated pear pieces were divided into 9 batches of 4 pieces each. Four batches were submitted to UV-C light treatment of 2.5, 5, 7.5 and 10 kJ/m^2, each. Two of the batches were used to study the effect of washings with AEW and NEW as decontaminants and another two sets of fruits were treated with SH solution and with DW. The washings treatments (AEW, NEW, SH and DW) occurred by dipping the fruits in flasks containing 500 mL of the treating solutions, during 5 min in agitation (150 rpm) in an orbital agitator. After the application of the treatment solutions, pear pieces were drained and rinsed with cold distilled water for 3 min at 150 rpm in an orbital shaker. Then, these four batches were left to dry in a laminar flow hood for 30 min.

The last inoculated batch of fresh-cut pear was not submitted to any decontamination treatment and was used as control.

In the case of fresh-cut pears inoculated with a bacterial mixture, *E. coli*, *S. enterica* and *Listeria* spp. were prepared as previously described to achieve a final concentration of 10^8 cfu/mL of each bacterium. The quantification of each microorganism was confirmed using the Miles and Misra method (1938), plating 20 µL drops of diluted cultures on Sorbitol MacConkey Agar for *E. coli*, and Hektoen Agar for *S. enterica* (incubation at 37±1 °C for 24±2 h) and on Palcam Agar for *Listeria* spp. (incubation at 37±1 °C for 48±2 h). Samples of pear pieces were inoculated by dipping into 500 mL of a mixture of the three bacteria and left to dry. Afterwards, EW and UV-C treatments, as well as SH and DW, were applied as previously described. Inoculated, but untreated samples were used as a control.

The evaluation of the population of each foodborne bacteria was determined in the pear samples after drying for 30 min. For each decontamination treatment, 10 g of pear pieces were transferred into sterile Stomacher bags and mixed with 90 mL of sterile saline peptone following a homogenization in a Stomacher, during 2 min, as previously described. Serial dilutions in saline peptone were made and 20 µL drops, in triplicate, were plated on the surface of the TSA medium using the Miles and Misra method (1938). Colonies were counted after incubation during 24±2 h at 37 °C, and the results expressed as log cfu/g of pears. For each treatment condition four replications were performed and the experiment was repeated twice.

2.7. Effect of UV-C irradiation and AEW and NEW on the quality parameters of fresh-cut pear

The effects of UV-C irradiation (2.5, 5, 7.5 and 10 kJ/m^2), AEW and NEW (100 mg/L of free chlorine), SH (100 mg/L of free chlorine) and distilled water (DW) on the quality parameters (color, soluble solid content, titratable acidity, pH and firmness) of fresh-cut pear were also studied. The quality parameters were measured, in triplicate, in pear pieces decontaminated with each treatment, 4 hours after the treatments when the fruit pieces submitted to washings were dried. Results were compared with determinations performed with untreated fresh-cut pear immediately after cutting (AC) and 4 hours after cutting, used as control (CK).

Surface color of pear pieces was evaluated with a CR-300 Minolta chromameter (Minolta, Inc., Tokyo, Japan), standardized against a white tile, using the CIE *L**, *a**, *b** parameters. The Hue angle was calculated from averaged *a** and *b**.

The soluble solid content (°Brix) (SSC) of fresh-cut pears was measured using a refractometer (Atago Co. Ltd. Tokyo, Japan) in the juice extracted from the pear pieces.

Titratable acidity (TA) was measured in 10 mL of pear juice dilute in 10 mL of distilled water and titrated with 0.1 N of NaOH (Merck, Darmstadt, Germany) to a pH value of 8.2. Results were calculated as g of malic acid per liter.

Firmness was determined using a texture analyzer (Chatillon, Chatillon Force TCD200, Digital Force Gauge Dfis 50 penetrometer, USA) with a 8 mm diameter plunger that penetrated 7 mm. Firmness was expressed in Newton (N).

2.8. Statistical analyses

The values of reduction in bacteria on pear pieces were calculated by subtracting the population of inoculated but untreated pears from the microbial population after treatment in the same storage conditions. Values represent the means of 2 different experiments, with 4 replicates per treatment per experiment. The quality parameters were determined in triplicate in samples decontaminated with each treatment. Data were subjected to analysis of variance and Duncan's multiple range tests using SPSS v.20.0 software (SPSS Inc., USA). Significant differences in reduction values were established by the least significant difference at the 0.05 level of significance.

3. Results and Discussion

3.1. Growth of E. coli, S. enterica and Listeria spp. on fresh-cut pears

The survival and growth of *E. coli* (Fig. 1A), *S. enterica* (Fig. 1B) and *Listeria* spp. (Fig. 1C) inoculated on fresh-cut 'Rocha' pears, at different temperatures (4, 8, 12 and 20 °C) during a

328 period of eight days are represented in Fig. 1. At 20 °C the population of the three foodborne
329 pathogens increased exponentially during approximately the first day, with maximum specific
330 growth rates of 2.98±0.258, 2.7±0.322 and 3.1±0.296 day^{-1}, for *E. coli*, *S. enterica* and *Listeria*
331 spp., respectively. At 12 °C a similar behavior was observed for the three microorganisms but
332 with maximum specific growth rates slightly lower of 1.9±0.193, 2.2±0.23, 2.6±0.636 day^{-1},
333 respectively. After the exponential growth a stationary phase occurred until the end of the
334 assays. An increase of initial viable populations, recovered from inoculated fresh-cut pears, of
335 6.0-6.2 log cfu/g to 8.1-8.6 log cfu/g, at the end of the study was observed.
336 At 8 °C, *E. coli* and *S. enterica* were able to grow exponentially during approximately 3 days at
337 maximum specific growth rates of 0.37±0.043 and 0.66±0.127 day^{-1}, respectively, although
338 more slowly than the temperatures of 12 and 20 °C. Then, a stationary phase growth was
339 observed until the 8^{th} day when final populations of 7.4±0.074 and 7.2±0.124 log cfu/g,
340 respectively, were counted. Regarding *Listeria* sp., an adaptation phase of 0.58±0.279 day was
341 estimated, which was followed by exponential growth at a rate of 0.89±0.113 day^{-1}, reaching the
342 stationary phase, approximately, after 3 days. Counts of the *Listeria* population increased from
343 6.2±0.040 log cfu/g, at the beginning, to maximum values of 8.5±0.1 log cfu/g of pear, at end of
344 the experiment.
345 At 4 °C the population of *E. coli* and *S. enterica* remained almost unchanged during the period
346 studied, after the inoculation moment, or slowly declined. In the case of *E. coli* a death rate of -
347 0.35±0.13 day^{-1} was calculated. In regards to the growth of *Listeria* spp. in fresh-cut pears at 4
348 °C, an adaptation phase of less than 24 h was estimated followed by an exponential growth at a
349 rate of 0.38±0.0567 day^{-1} reaching a population of 8.1±0.102 log cfu/g.
350 The results described for pear are similar to previous research regarding the growth of
351 foodborne pathogens in cut fruit at the temperatures tested. For example, *E. coli* O157:H7, *S.*
352 *enterica* and *L. innocua* were able to grow exponentially at temperatures of 20 and 25 °C on
353 fresh-cut peaches of different varieties (Alegre et al., 2010b) and on fresh-cut apples 'Golden
354 delicious' (Alegre et al., 2010a). At 10 °C these microorganisms were able to grow on the fruits
355 reaching lower populations while at 5 °C, only *L. innocua* was able to multiply. *E. coli*

O157:H7 also showed an exponential growth in minimally processed melon at 25 ºC but was unable to grow on pineapple at 25 and 5 ºC (Abadias et al., 2012). Strawn and Danyluk (2010) observed a similar behavior of *E. coli* O157:H7 and *S. enterica* on cut papayas and mangos at 23 ºC. At 12 ºC only *Salmonella* grew on both fruits and *E. coli* was only able to grow on papayas. The same authors observe that both enterobacteria did not grow on the fruits at 4 ºC but were able to survive during 28 days.

The differences in the growing capacity of bacteria on the fruits may be explained by intrinsic characteristics of the fruits' tissues, including pH, composition, presence/absence of inhibitor compounds and by the physiologic capacity of the different microbial species to adapt to eventual stressful conditions. In the case of peaches, for example, the highest populations of foodborne bacteria registered were obtained in the varieties with the highest pH values (4.12 and 4.73) (Alegre et al., 2010b) and on fresh-cut strawberries (pH 3.6-3.8). Flessa, Lusk and Harris (2005) and Knudsen, Yamamoto and Harris (2001) reported that *E. coli*, *S. enterica* and *L. monocytogenes* were not able to grow. The results presented indicate that fresh-cut pears are a good substrate for the three pathogens to survive and grow at temperatures above 8 ºC while at 4 ºC, only *Listeria* spp. was able to grow after a 24 h adaptation phase. Fresh-cut pear has a pH tissue value of 5.28 which is slightly acidic for a fruit and has a low titratable acidity of 1.3 g malic acid/g, when compared to other fruits (peaches- 4.1-8.9 g malic acid/l; apples-2.16-8.2 g malic acid/l).

Storage temperature is one of the main factors regulating the microbial growth in the food matrices. *Listeria* is a psychrotrophic microorganism and when at refrigeration temperatures induces a complex mechanism of adaptation, the "cold shock response", that allows it to rapidly adapt and multiply reaching dangerous populations enough to cause disease during the shelf-life of food (Melo, Andrew & Faleiro, 2015). On the other hand, many microorganisms in environments where pH is lower than optimal developed a number of alterations, involving the activation of a number of genes. For example, cells may alter the external pH value by expressing enzymes whose function is to raise external pH, such as lysine decarboxylase, in *Salmonella*, which converts lysine to cadaverine, an alkaline substance, arginine decarboxylase

384 in *E. coli* (Beales, 2004) and arginine deiminase in *L. monocytogenes* (Melo et al., 2015).
385 Exposure to mildly acidic conditions induces tolerance mechanisms, such as the acid tolerance
386 response (ATR) described in the foodborne microorganisms *S. enterica* and *E. coli* (Foster,
387 2001) and *L. monocytogenes* (Melo et al., 2015). These mechanisms, among others, enable the
388 bacteria to survive on food products such as fruits, with a pH lower than the microbial optimal
389 pH, and protect them from subsequently more severe pH/acid conditions. Microorganisms may
390 evolve to being able to rapidly adapt and tolerate/resist a particular stress. This adaptation or
391 resistance will allow the survival and growth of foodborne microorganisms, thus having great
392 implications on the safety of food products, such as acidic food stored at low temperatures.
393
394 *3.2. Inactivation of E. coli, S. enterica and Listeria spp. (individually and in a mixture) on fresh-*
395 *cut pears using UV-C irradiation and AEW and NEW*
396
397 The antimicrobial activity of UV-C irradiation at different doses (2.5, 5, 7.5 and 10 kJ/m^2) and
398 electrolyzed water (AEW and NEW) (100 mg/L of free chlorine), on fresh-cut pears inoculated
399 with single cultures of *E. coli*, *S. enterica* and *Listeria* spp. is represented in Fig. 2 and with a
400 mixture of the three groups of microorganisms, in Fig. 3. The results were compared with fresh-
401 cut fruit treated with SH solution (100 mg/L of free chlorine) and distilled water (DW).
402
403 The exposure of pear pieces to the different doses of UV-C irradiation and EW, as
404 decontaminants, induced reductions in the populations of the three foodborne pathogens studied.
405 In the case of *E. coli* population (in a single culture) the reductions obtained ranged from 2.3 log
406 cfu/g to 3.4 log cfu/g after the application of UV10 and UV7.5, respectively ($p<0.05$) (Fig. 2).
407 When *E. coli* was inoculated in the pear with a mixture of species, the most efficient treatment
408 was also UV7.5 resulting in the highest reduction values of *E. coli* population of 3.2 log cfu/g
409 (Fig. 3). None of the UV-C treatments resulted in microbial reductions inferior to 1.97 log cfu/g.
410 Regarding EW washings, microbial decreases values of 0.53 to 1.1 log cfu/g were achieved and

411 no significant differences among the bacterial population drops obtained in samples washed
412 with AEW, NEW or SH were observed ($p>0.05$), whether in a single culture or in a cocktail
413 (Fig. 2 and Fig. 3). The microbial reductions obtained with decontaminations of AEW and
414 NEW showed no differences from the results achieved with washings of SH solutions ($p>0.05$).
415
416 The application of UV-C irradiation on fresh-cut pear inoculated with *S. enterica* in a single
417 culture led to the highest reductions of this microorganism when doses of UV10 and UV7.5
418 were applied with values of 2.4 and 2.4 log cfu/g, respectively and no statistical differences
419 were found between them ($p>0.05$) (Fig. 2). In the mixed culture, the UV7.5 was also the
420 treatment that allowed the higher reduction values (2.8 log cfu/g) for *S. enterica* (Fig. 3). None
421 of the UV-C treatments resulted in the reduction level of *S. enterica* inferior to 1.9 log cfu/g.
422 Washing the contaminated pears with AEW and NEW caused a decrease in the levels of *S.*
423 *enterica* population of 0.92 and 1.1 log cfu/g in a single culture (Fig. 2) and of 0.76 and 0.67 log
424 cfu/g in a mixed culture (Fig. 3). In both cases, the results obtained in the decontaminations with
425 AEW and NEW showed no differences from the disinfections performed with SH ($p>0.05$). The
426 washing with DW was the treatment that resulted in lowest reduction values, of *E. coli*, *S.*
427 *enterica* and *Listeria* spp. populations on the fresh-cut pears.
428
429 With regards to *Listeria* spp. in a single culture inoculation, the highest reductions were
430 achieved when the UV10 treatment (3.3 log cfu/g) and UV7.5 (2.9 log cfu/g) were applied and
431 no statistical differences between these results were detected ($p>0.05$). The lowest microbial
432 reduction of 1.7 log cfu/g was caused by UV2.5 (Fig. 2). When the fresh-cut pears were
433 inoculated with a mixture of the three pathogens, the highest reduction (2.4 log cfu/g) of
434 *Listeria* spp. was obtained with the UV7.5 treated samples, although there were no statistical
435 differences from UV5 treated pears (2.1 log cfu/g) ($p>0.05$). The lowest reductions were
436 observed with the UV10 (1.5 log cfu/g) for *Listeria* spp. (Fig. 3). Concerning the utilization of
437 EW as a decontaminant, no significant differences were observed among the microbial
438 reductions achieved in the pear samples washed with AEW and NEW, which caused a decrease

439 in *Listeria* spp. values of 1.1 and 1.03 log cfu/g, in a single culture (Fig. 2), and 1.1 and 0.92 log
440 cfu/g in the mixture (Fig. 3), respectively. Washing with SH resulted in higher reduction values
441 of *Listeria* spp. population than those caused by the utilization of AEW and NEW, when
442 inoculated in a single culture but not in a mixed culture.
443
444 According to the results obtained, *E. coli*, *S. enterica* and *Listeria* spp. populations were
445 significantly reduced in fresh-cut pear by UV-C and EW treatments. The UV7.5 appeared to be
446 the most efficient decontamination method, as its application resulted in the decreasing of the
447 three foodborne populations of pathogens higher than 2.4 log cfu/g when inoculated in a single
448 or in a mixed culture. Additionally, as can be observed in Fig. 2 and Fig. 3 the application of
449 higher doses of UV-C than UV7.5 did not always result in higher microbial load reductions.
450 These results may be explained by the fact that the higher UV-C doses may eventually induce
451 chemical or physical changes in the fruit tissues that could result in the protection of the
452 microorganisms from the incidence of the radiation or increasing their resistance mechanisms.
453 For example, Schenk et al. (2008) cite that the presence of solids in the fruit matrix or the fruit
454 surface topography may block the microbial cells from receiving the UV-C rays.

455 The UV-C decontaminations were more effective than the ones performed with SH which
456 resulted in reductions less than 1 log cfu/g with exception of *E. coli* and *Listeria* (when
457 inoculated in a single cultures). Regarding EW decontaminations, the level of microbial
458 reductions achieved did not exceed 1.1 log cfu/g. EW decontaminations resulted in lower
459 microbial reductions compared to those obtained when the UV-C was applied, although they
460 were not significantly different from the decontaminations performed with SH. Previous studies
461 conducted by Syamaladevi et al. (2013) to evaluate the effect of UV-C on pear (Fresh D'Anjou
462 cv) decontamination achieved reduction values of *E. coli* population of 3.7 log cfu/g on the
463 surface of intact fruits and 3.1 log cfu/g on wounded fruits using UV-C irradiation at the dose
464 7.56 kJ/m^2. Jemmi et al. (2014) observed that the dose 6.22 kJ/m^2 was more effective than 8.3
465 kJ/m^2 in reducing yeasts and molds and the total mesophilic on palm dates. The effectiveness of

466 UV-C radiation in the inactivation of *E. coli*, *L. innocua* and *S. enterica* were also observed on
467 apples (1.0 kJ/m^2) (Graça e al., 2013) and of *E. coli* O157:H7 and different serotypes of *S.*
468 *enterica* in apricots (Yun et al., 2013). Yaun, Sumner, Eifert and Marcy (2004) used UV-C light
469 to inactivate the population of *E. coli* and *S. enterica* on lettuce, tomato and apple surfaces and
470 observed that the UV-C was more effective against these bacteria than SH (20-320 ppm).
471 Additionally, in Yale pear the utilization of UV-C radiation at dose 5 kJ/m^2 was successfully
472 used to inhibit the growth of *Monilinia fruticula* as well as enhance the activity of some
473 antioxidant enzymes and thus contributing to the decrease of the application of chemical
474 fungicides (Li, Zhang, Cui, Yan, Cao, Zhao, & Jiang, 2010). When comparing UV-C with EW
475 decontaminations, Kim and Hung (2012) reported that UV-C treatments were more effective
476 than EW inactivating *E. coli* O157:H7 in blueberries. In the present study, decontamination of
477 pears with AEW, NEW and SH were less effective on the bacterial reduction than was UV-C
478 irradiation. This is in agreement with the results presented by Kim and Hung (2012).
479 Nevertheless, unlike the results of Graça et al. (2011) AEW was not more efficient than NEW in
480 reducing the level of *E. coli*, *S. enterica*, *L. innocua* in pear as it was in apple. The reaction of
481 chlorine with the organic components of cut fruits has been used to explain its low activity due
482 to the lowering of its effective concentration before damaging microorganisms (Graça et al.,
483 2011). The fact that AEW and NEW showed equal disinfection efficacy than SH indicates that
484 these techniques can be used as an alternative to SH, as they are safer and do not present great
485 health/environmental problems compared to NaClO. Additionally, the effect of the different
486 decontaminations on pear pieces was not affected by the total population size since the
487 microbial reductions achieved on the samples inoculated with a combination of the three groups
488 of microorganisms was similar to that inoculated with only one group of bacteria. This has been
489 reported in other studies, such as in apples (Graça et al., 2011) and different vegetables (Abadias
490 et al., 2008).
491
492 The high/low effectiveness of physical or chemical treatments on food decontamination are
493 highly dependent on food surface properties such as hydrophobicity, electric charge and

roughness, which may influence the adhesion and microbial distribution of food surfaces (Araújo, Andrade, Mendes da Silva, de Carvalho, Sa Silva & Ramos, 2010). Additionally, hydrophobic/hydrophilic interactions between surfaces and bacteria are determinant in the process of adhesion/attachment and posterior inactivation of microbial cells through the various decontamination methods. These aspects certainly affect the difficulty of removing or inactivating microorganisms by chemical or physical agents and may explain the different levels of microbial reduction obtained by UV-C, AEW, NEW and SH in the diverse matrices. However, although the antimicrobial effect of UV-C irradiation is dependent on the dose applied, food surface characteristics (roughness, hydrophobicity), initial bacterial inoculum, bacterial type and the low penetration capacity, it revealed to be more effective as a decontaminant of fresh-cut pear than the chemical sanitizers used (SH, AEW and NEW). The origin of the microbial food contamination (equipment, handler and washing water contamination, among others) is another important aspect when selecting the most adequate method of disinfection.

3.3. Effect of UV-C irradiation and AEW and NEW on the quality parameters of fresh-cut pear

The effect of the UV-C and electrolyzed water (used in the antimicrobial studies described earlier) on the quality parameters of fresh-cut pear was studied before and after the application of the decontamination treatments. For this purpose, color, titratable acidity (TA), pH, soluble solid content (SSC) and firmness were measured on samples submitted to the different treatments and compared with the measurements of untreated samples immediately after cutting (AC) and 4 hours after cutting (CK). The results are shown in Table 1.

Table 1. 'Rocha' fresh-cut pear quality parameters (L^*, H^0, soluble solid content (SSC), titratable acidity (TA), pH and Firmness) after treating with UV-C irradiation (2.5, 5, 7.5 and 10 kJ/m^2) and after washing with acidic electrolyzed water (AEW), neutral electrolyzed water (NEW), sodium hypochlorite (SH) (100 mg/L of free chlorine), and with distilled water (DW). Untreated samples were used as control, right after cutting (AC) and 4 h after cutting (CK). For each value (± standard error) different letters (a, b, c, d) indicate signficant differences ($p < 0.05$) between treatments according to Duncan multiple range test.

524

Treatment	Quality Parameter					
	*L**	H^0	SSC	TA	pH	Firmness
AC	96.20 (±0.57)a,b	-0.14 (±0.51)a	14.90 (±0.42)b	1.30 (±0.04)c	5.28 (±0.13)d	56.80 (±2.89)a
CK	96.40 (±1.18)a,b	0.93 (±0.54)a	14.30 (±0.15)b	0.94 (±0.04)a	5.15 (±0.18)b,c	65.80 (±4.97)a
UV2.5	96.93 (±1.10)a,b	1.45 (±0.10)a	13.70 (±0.75)b	0.98 (±0.08)a,b	4.50 (±0.13)a,b	61.60 (±7.62)a
UV5	95.88 (±1.61)a	1.52 (±0.02)a	13.13 (±1.18)b	1.14 (±0.04)a,b,c	5.06 (±0.13)b,c,d	60.87 (±3.74)a
UV7.5	98.59 (±1.03)b	1.41 (±0.08)a	14.50 (±1.2)b	1.16 (±0.02)a,b,c	4.80 (±0.24)a,b,c,d	64-00 (±5.62)a
UV10	97.54 (±0.92)b	1.46 (±0.03)a	12.37 (±0.64)a,b	1.09 (±0.08)a,b,c	4.85 (±0.24)a,b,c,d	67.17 (±3.74)a
AEW	97.94 (±0.94)b	0.42 (±0.99)a	11.90 (±0.95)a,b	1.34 (±0.15)c	4.65 (±0.31)a,b,c	54.93 (±8.85)a
NEW	93.59 (±1.17)a	0.46 (±0.95)a	14.47 (±1.05)b	1.14 (±0.10)a,b,c	4.40 (±0.07)a	55.93 (±6.53)a
SH	102.22 (±0.26)c	1.31 (±0.04)a	9.80 (±1.67)a	1.09 (±0.11)a,b,c	4.78 (±0.15)a,b,c,d	64.40 (±7.96)a
DW	96.96 (±1.22)a,b	0.90 (±0.59)a	14.83 (±0.23)b	1.23 (±0.04)b,c	4.62 (±0.03)a,b,c	55.87 (±3.43)a

525

526

527 The decontamination of pears using the different treatments did not induce changes in the
528 parameter *L** ($p>0.05$) with the exception of pear treated with SH, where an increase in *L** was
529 observed, meaning the color of the fruit surface became lighter after the SH washing ($p<0.05$).
530 However, regarding Hue, no statistical differences were detected, among the samples ($p>0.05$).
531 The value of the SSC (°Brix) was not affected by the decontamination treatments applied to the
532 fresh-cut pears with the exception of the SH washing that caused a significant decrease in its
533 value (from 14.3±0.15 in the CK to 9.8±1.67 in the SH washed pear) ($p<0.05$). This result may
534 be explained by the fact that chlorine reacts with the organic material of the pear, resulting in a
535 decreasing of some substances such as the sugars.
536 In regards to TA, a significant decrease of its value from 1.3 g malic acid/L pear juice to 0.94 g
537 malic acid/L pear juice was observed when comparing the measurements performed in untreated
538 pears immediately after cutting (AC) with the untreated pears analyzed 4 hours after cutting
539 (CK) ($p<0.05$). However, there were no statistical differences among the CK and treated pears
540 acidity values with the exception of AEW and DW washed pears. In the case of the pH, a
541 decrease in its value was observed when comparing the measurements performed in untreated
542 pears, immediately after cutting (AC), with the untreated pears analyzed 4 hours after cutting
543 (CK) ($p<0.05$) (from 5.28±0.13 to 5.15±0.18). Except for pears washed with NEW, no
544 differences were found among the pH value of fresh-cut pear treated when compared with the

CK. Additionally, there were no significant differences in firmness among the different decontaminated treated fresh-cut pear ($p>0.05$). Several studies reported that UV-C radiation did not affect the quality parameters of fresh-cut fruits. In a study conducted by Graça et al. (2013) on fresh-cut apples submitted to UV-C was observed that color, SSC and acidity were not significantly different after the treatment. Manzocco et al. (2009a, 2009b) also did not observe significant differences in color and firmness of fresh-cut melons and fresh cut apples treated with UV-C, respectively. Regarding electrolyzed water, data obtained by Jia, Shi, Song and Li (2015) with Chinese yam indicate that these chemical decontaminants may have a protecting effect on the color of the yam.

4. Conclusion

Minimally processed 'Rocha' pear (pH 5.28 and titratable acidity 1.3 g malic acid/L) has shown to be a good substrate for the survival and growth of *E. coli*, *S. enterica and Listeria* spp. The populations of *E. coli*, *S. enterica* and *Listeria* spp. were significantly reduced in fresh-cut pear after the application of UV-C and EW decontamination technologies. The use of UV-C resulted in microbial reductions higher than 2 log cfu/g while AEW, NEW and SH resulted in reductions of approximately 1 log cfu/g. In general, the UV-C dose of 7.5 kJ/m^2 caused the highest microbial reduction. UV-C and EW seem to be promising decontamination technologies as they allow the reduction of foodborne bacteria population and the amount of SH without greatly affecting the quality of fresh-cut pear. However, alone, none of them completely eliminate the pathogenic bacteria thus alerting the necessity for a strategy that combines different technologies in order to increase the safety of fresh-cut fruit. The results highlight the importance of preventing contamination and cross contamination, selecting an adequate decontamination technology and of maintaining a strict temperature control from production and processing until consumption of fresh-cut pear.

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Fig. 1. Growth of inoculated bacteria in pear pieces stored for 8 days at 4°C, 8°C, 12°C and 20°C. (A*) Escherichia coli*; (B) *Salmonella enterica*; (C) *Listeria* spp.. Values are the means of 2 experiments with 4 replicates each and bars indicate standard error.

(◊ 4 °C; □ 8 °C; ● 12 °C; O 20 °C)

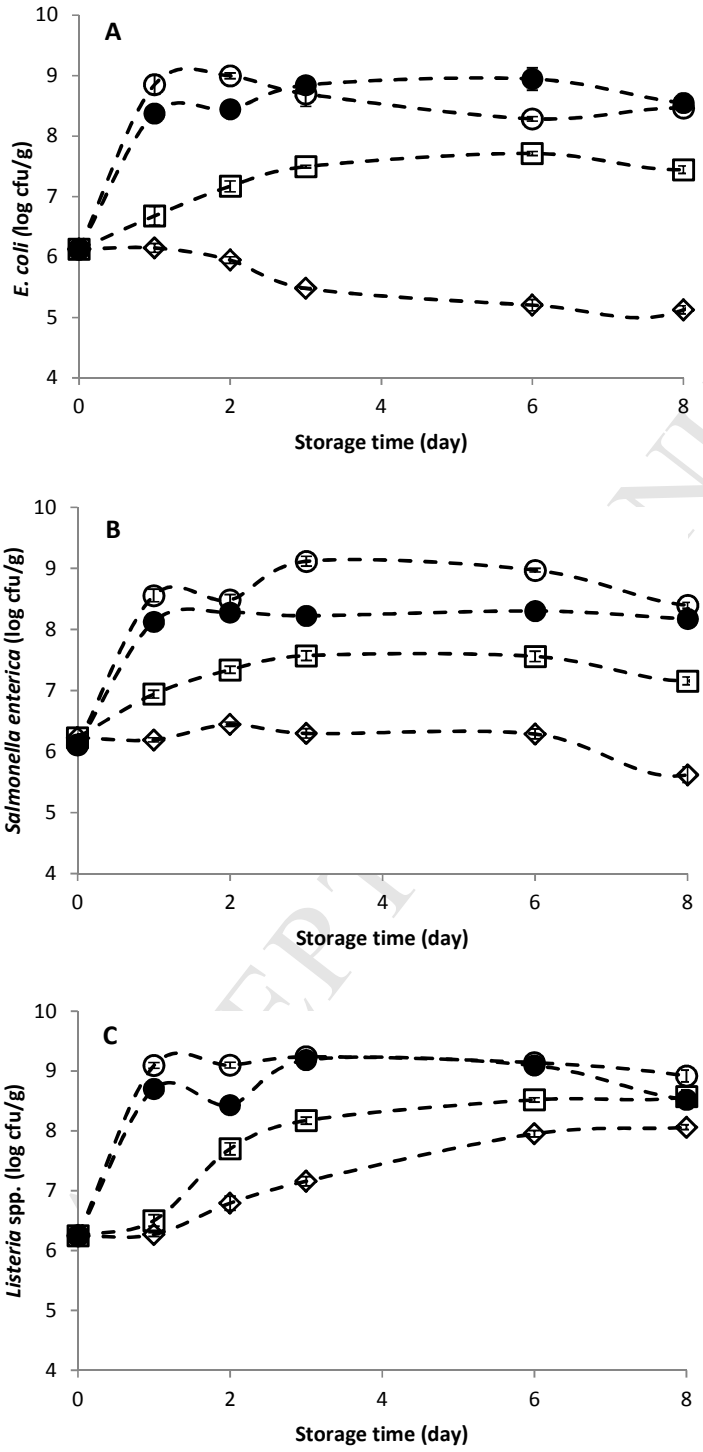

Fig. 2. Reduction of *E. coli*, *S. enterica* and *Listeria* spp. (individually) after treating pears slices with UV-C illumination, acidic electrolyzed water (AEW), neutral electrolyzed water (NEW), sodium hypochlorite (SH) (100 mg/L of free chlorine) and with distilled water (DW). For each pathogen, columns with different letters indicate significant differences between treatments using Duncan multiple range test (P < 0.05%). Values are the means of 2 experiments with 4 replicates each and bars indicate standard errors. (■ *E. coli*; ■ *S. enterica;* ■ *Listeria* spp.).

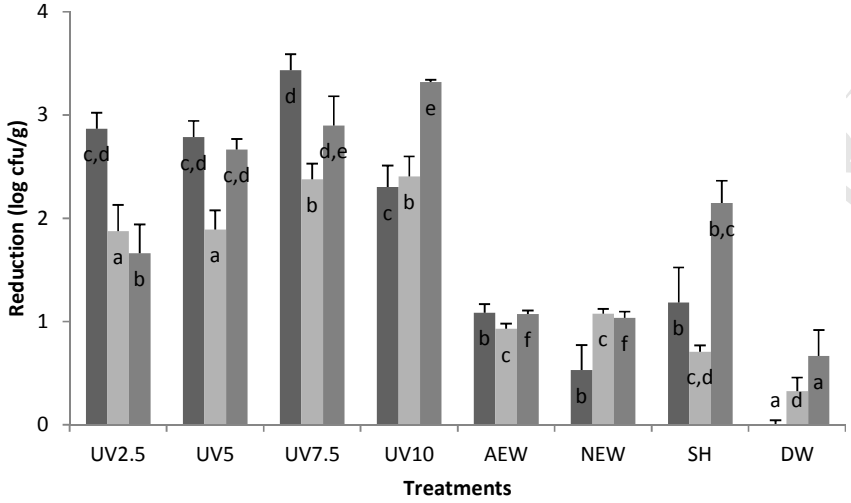

Fig. 3. Reduction of *E. coli*, *S. enterica* and *Listeria* spp. (mixture of the 3 bacteria) after treating pears slices with UV-C illumination, acidic electrolyzed water (AEW), neutral electrolyzed water (NEW), sodium hypochlorite (SH) (100 mg/L of free chlorine), and with distilled water (DW). For each pathogen, columns with different letters indicate significant differences between treatments using Duncan multiple range test ($P < 0.05\%$). Values are the means of 2 experiments with 4 replicates each and bars indicate standard errors (*E. coli*; *S. enterica; Listeria* spp.).