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RESEARCH ARTICLE

Inactivation of *Salmonella enterica* ser. Poona and *Listeria monocytogenes* on fresh-cut "Maradol" red papaya (*Carica papaya* L) treated with UV-C light and malic acid

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Abstract In recent years, the consumption of fresh-cut fruit has been associated with outbreaks of diseases caused by different emerging pathogenic microorganisms. Therefore, great efforts to find methods that ensure the quality and safety of minimally processed products are needed. The main objective of this study was to evaluate the effect of different doses of UV-C light (0, 0.96, 2.88, 5.76 and 8.64 kJ/m^2) and treatments with malic acid (0, 0.5, 1.0 and 1.5 %, w/v) applied into dipping solutions containing calcium lactate (1 %, w/v) and ascorbic acid (0.5 %, w/v) on the inactivation of *Salmonella enterica* ser. Poona and *Listeria monocytogenes* inoculated onto fresh-cut "Maradol" papaya (0.7 × 3.5 × 2.5 cm). Significant differences ($p \leq 0.05$) in counts of *Salmonella* Poona and *L. monocytogenes* on fresh-cut papaya, treated with different doses of UV-C light and different concentrations of malic acid, were observed. A synergistic effect between malic acid (1.5 %) and UV-C light (8.64 kJ/m^2) was noted. Reductions of 5.28 and 3.15 Log_{10} CFU/g for *Salmonella* Poona and *L. monocytogenes*, respectively, were reached using this combination. In conclusion, the combination of dipping solutions containing malic acid and treatment with UV-C light could be a good alternative to ensure the safety of minimally processed papayas.

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1 Introduction

Raw fruits may be contaminated with pathogenic and spoilage microorganisms during their growing in fields, orchards or greenhouses, or during harvesting, post-harvest handling and/or distribution (Beuchat 2002). Fresh fruits have a natural protective barrier (skin) that can act effectively against several spoilage and pathogenic microorganisms; however, during processing (peeling and cutting) this protection is eliminated. Ready-to-eat fresh-cut fruits are exposed to unfavorable environmental conditions during handling, packaging and storage (Brackett 1994; Martín-Belloso and Rojas-Graü 2005). This fact may be related to the great number of documented outbreaks of human infections associated with consumption of fresh-cut fruits (CDC 2011). Recently, a multistate outbreak of humans infected by *Salmonella enterica* was linked to fresh imported whole papayas. A total of 106 individuals infected with this strain were reported from 25 states of USA (CDC 2011). Populations of *Listeria monocytogenes* have also been isolated from fresh papaya (Vahidy et al. 1992). Penteado and Leitao (2004) and Fernández-Escartín et al. (1989) have demonstrated that *Salmonella* spp. and *L. monocytogenes* populations can survive and/or grow on pulp and fresh-cut papaya. Therefore, if these products are not hygienically and adequately handled and processed, then they might be hazardous to the health of the consumers.

To maintain the safety in these kinds of products, a great number of alternative technologies individually

or in combination are being used; among them, radiation with UV-C light (Gonzalez-Aguilar et al. 2010; Corbo et al. 2010). Treatment with ultraviolet energy could offer several advantages to fresh-cut fruit processors as it does not leave any residue, and does not have legal restrictions, it is easy to use and lethal to a wide broad of microorganisms, and does not require high economic investment nor the implementation of expensive safety equipment (Rivera-Pastrana et al. 2007). This type of radiation has its peak emission at 254 nm, and has high germicidal action because it is able to cause damage in the DNA of microorganisms, blocking the cell replication; and if damage is not repaired, cell death may occur (Rivera-Pastrana et al. 2007; Gómez et al. 2010). This technology has been applied on fresh-cut melon, mango, apple, watermelon and papaya to prolong their shelf-life (Lamikanra et al. 2002; Gonzalez-Aguilar et al. 2006; Fonseca and Rushing 2006; Gómez et al. 2010; Artés-Hernández et al. 2010; Corbo et al. 2010; Calderón-Gabaldón et al. 2012); however, their effects on pathogenic microorganisms on fresh and fresh-cut fruits has been investigated by just a few studies (Yaun et al. 2004).

Organic acids such as malic, citric and lactic acid have been used and approved by different countries as antimicrobial agents in fresh fruits and their derivatives to control the growth of foodborne bacteria such as *Salmonella* spp. and *L. monocytogenes*. In fact, some of these organic acids have been recommended to be used in fresh-cut apples, pears, melon and mango to inactivate *Salmonella* Enteritidis, *L. monocytogenes* and *E. coli* O157: H7 and, to inhibit the natural microflora (Millán et al. 2001; Di-Persio et al. 2003; Derrickson-Tharrington et al. 2005; Cordeiro et al. 2005; Raybaudi-Massilia et al. 2007, 2009b; Calderón-Gabaldón et al. 2012). A detailed discussion about the antimicrobial activity and mechanisms of action of organic acids are provided in the review of Raybaudi-Massilia et al. (2009a).

The objective of this study was to inactivate populations of *Salmonella* Poona and *L. monocytogenes* inoculated on fresh-cut "Maradol" papaya red by dipping solutions containing malic acid and treated with UV-C light, as hurdle technology.

2 Materials and methods

2.1 Fruit characterization

Papayas (*Carica papaya* L) cv. "Maradol" red partially ripe, were provided by TAFAYET C.A. (Caracas, Venezuela). Total acidity (expressed as grams of malic acid per 100 g of papaya), pH (Microprocessor pH meter, Hanna Instruments, Romania), and percentage of soluble solids (RX-1000 refractometer, Atago Company Ltd., Tokyo, Japan) were measured according to official methods for fruit juices and other vegetables and derivatives COVENIN 924-83 (1983); COVENIN 1151-77 (1977). Firmness (TA-TX2i texture analyzer, Stable Micro Systems Ltd., Godalming, Surrey, UK) was also measured to characterize the fruit (Table 1).

2.2 Microorganisms

Strains of *Salmonella enterica* ser. Poona (CVCM 1921) and *Listeria monocytogenes* (CVCM 449) were provided by the "Centro Venezolano de Colecciones de Microorganismos (CVCM)" of the Institute of Experimental Biology of the Central University of Venezuela, Caracas-Venezuela for this study. Pure cultures of *Salmonella* Poona were grown in trypticase soy broth (HIMEDIA, Mumbai, India) at 37 °C with continuous agitation for 8 h at 100 rpm; whereas *L. monocytogenes* populations were grown in trypticase soy broth with 0.6 % (w/v) yeast extract (Merck, Darmstadt, Germany) at 37 °C without agitation for 24 h. These parameters of incubation were performed for obtaining cells of both microorganisms in early stationary phase (10^9 CFU/ml) from growth curves previously made in the laboratory (data not shown).

2.3 Dipping solutions

Extra pure pent-hydrated calcium lactate (Quimitec C.A., Maracay, Venezuela) at 1 % (w/v) and ascorbic acid (Quimitec C.A) at 0.5 % (w/v) were used as stabilizing agents of texture and color, respectively, in combination with extra pure DL-malic acid (Quimitec C.A) at 0, 0.5 %; 1 and 1.5 % (w/v), as antimicrobial agent. Samples treated with distilled water alone and treated with stabilizing agents of texture and color as

Table 1 Physicochemical properties of fresh-cut "Maradol" papaya

Parameters	Partially ripe papaya
pH	5.5 ± 0.01[a]
Soluble solids (%)	10.2 ± 0.1[a]
Total acidity (malic acid g/100 ml)	0.166 ± 0.20[a]
Firmness (*N*)	9.87 ± 1.28[b]

[a] Mean of three analysis ± SD

[b] Mean of ten analysis ± SD

controls were also considered. Concentrations of stabilizing agents and malic acid were selected according to the previously reported by Calderón-Gabaldón et al. (2012) for avoiding lost of physicochemical and sensory quality, and extending the microbiological shelf-life of fresh-cut papayas.

2.4 Sample preparation and inoculation

Papayas were selected according to weight, size, and uniform ripeness (partially ripe or commercial maturity), free from damage and physical defects. They were washed with a solution of sodium hypochlorite (200 ppm) for 5 min, rinsed with filtered tap water and, finally dried with absorbent paper. Then, fruits were peeled and cut into slices with a knife, and then with a rectangular hollow instrument of stainless steel into pieces of 0.7 × 3.5 × 2.5 cm. The pieces were immediately treated for 1 minute with a solution containing: calcium lactate (1 %, w/v) and ascorbic acid (0.5 %, w/v) with malic acid at different concentrations (0, 0.5, 1, 1.5 % w/v) or distilled water alone (control), at room temperature (25 °C), at a ratio fruit:solution = 1:2, and constant agitation using a hot plate magnetic stirrer (Corning PC-420, Scientific Support, Inc., California, USA). The liquid in excess was naturally drained at room temperature by approx. 5 min. Afterwards, 25 g (six pieces or slices) of treated fresh-cut papaya were individually inoculated on a side with 250 μL of pure cultures of *Salmonella* Poona or *L. monocytogenes* at a level of 10^7 CFU/mL on their surfaces using a sterile micropipette. The inoculum on the fresh-cut papayas was dried at room temperature for 10 min. Then, pieces inoculated were introduced into Ziploc® bags of single polyethylene layer (Oxygen permeability of 17,000 cc/m^2/24 h at 20 °C) (two bags per each treatment). The inoculation on fresh-cut papayas was made after treated in dipping solutions in order to avoid reductions of inoculated microbial populations by rinsing.

2.5 Treatment with ultraviolet-C light

Samples of fresh-cut papaya previously treated with dipping solutions and inoculated with *Salmonella* Poona or *L. monocytogenes* and put into Ziploc® bags were submitted to irradiation with UV-C light. Two sources of germicidal lamps of 15 W (Osram/Sylvania G15T8, Danver, Massachusetts, USA) were placed in the top and bottom of a chamber of treatment (Institute of Food Science and Technology, Central University of Venezuela, Caracas, Venezuela) at a distance of 15 cm of the samples. To assure a homogeneous distribution of the emitted light, the chamber inside was covered with a protective reflecting layer. Finally, Ziploc® bags (with a 63.05 % of transmission of UV-C irradiation) containing treated fresh-cut papayas were irradiated by both sides with different doses, calculated by the following equation (Yaun et al. 2004):

$$\text{UV dose}\,(\text{kJ/m}^2) = \text{irradiation}\,(\text{kJ/m}^2\text{s}) \times \text{exposure time}\,(\text{s})$$

where, the irradiation (1.6×10^{-2} kJ/(m^2s)) was calculated based on an average of 10 readings (taking account the percentage of transmission through the Ziploc® bag) using a portable radiometer / photometer (International Light, model IL400A, Massachusetts, USA). Exposure time of UV-C light was 0, 1, 3, 6 and 9 min equivalent to 0.00, 0.96, 2.88, 5.76 and 8.64 kJ/m^2, respectively.

Doses of UV-C light were chosen according to previous studies reported by Yaun et al. (2004) and Calderón-Gabaldón et al. (2012) for controlling pathogenic and spoilage microorganisms on fresh-cut fruit, respectively, without significantly affecting their physicochemical and sensory properties.

2.6 Experimental design

Different conditions of treatments were applied to fresh-cut papayas, based on a multi-level factorial design 5 × 5 × 2 (5 UV-C light doses, 5 dipping conditions and 2 repetitions) (Fig. 1), in order to evaluate the effect of two factors, dose of UV-C light and dipping condition on the inactivation of two pathogenic microorganisms such as *Salmonella* Poona and *L. monocytogenes*. The design consisted of 50 runs by each microorganism. Two bags by each treatment condition were evaluated ($n = 4$). The treatments were randomly carried out to avoid lurking variables. The experimental design was obtained using the statistical program Statgraphics Centurion XV version 15.1.02 statistical package (StatPoint, Inc., Warrenton, VA, USA).

2.7 Microbiological analysis

Ziploc® bags with 25 g of fresh-cut papaya treated and inoculated were diluted in 225 mL of phosphate buffer (pH 7–7.2), and left during 20 min at 35–37 °C for recovering of injured cells. Then, the samples were spread plated on Hektoen (Merck) agar for counting of *Salmonella* Poona and, PALCAM (HiMedia) agar for *L. monocytogenes* counts. Spread plated was made in duplicated and incubated for 24 and

48 h, respectively, at 37 °C. The microbial counts were analyzed immediately after the treatments, and reported as Log_{10} CFU/g. The recovery time (20 min) was selected taking into account the generation time of each microorganism from growth curves previously obtained in the laboratory (data not shown); where repairing of injured cells without cellular multiplication was assumed.

2.8 Statistical analysis

Results were analyzed with the statistical program Statgraphics Centurion XV version 15.1.02 statistical package (StatPoint, Inc.). Analysis of variance (ANOVA) with posterior multiple range test (MRT) were applied to find significant differences ($p < 0.05$) between microbial counts by UV-C light doses for each treatment condition and pathogenic microorganism.

3 Results and discussion

Slight reductions of populations of *Salmonella* Poona (about 1 Log_{10} CFU/g) were observed in fresh-cut papayas not treated with UV light, and dipped in distilled water (Fig. 2a; UV-C light doses = 0 kJ/m^2) or in a solution of calcium lactate (1 %) and ascorbic acid (0.5 %) without malic acid (0.0 %) (Fig. 2b; UV-C light doses = 0 kJ/m^2); whereas, populations of *L. monocytogenes* were not significantly ($p > 0.05$) affected under the same treatment conditions. Raybaudi-Massilia et al. (2009b) did not observe significant ($p > 0.05$) decreasing of *Salmonella* Enteritidis and *L. monocytogenes* populations on fresh-cut pears dipped into aqueous solutions containing calcium lactate (1 %), *N*-acetyl-ʟ-cysteine (1 %) and glutathione (1 %) just after inoculation. Lanciotti et al. (2003) demonstrated that fresh-cut apples dipped in 0.2 %

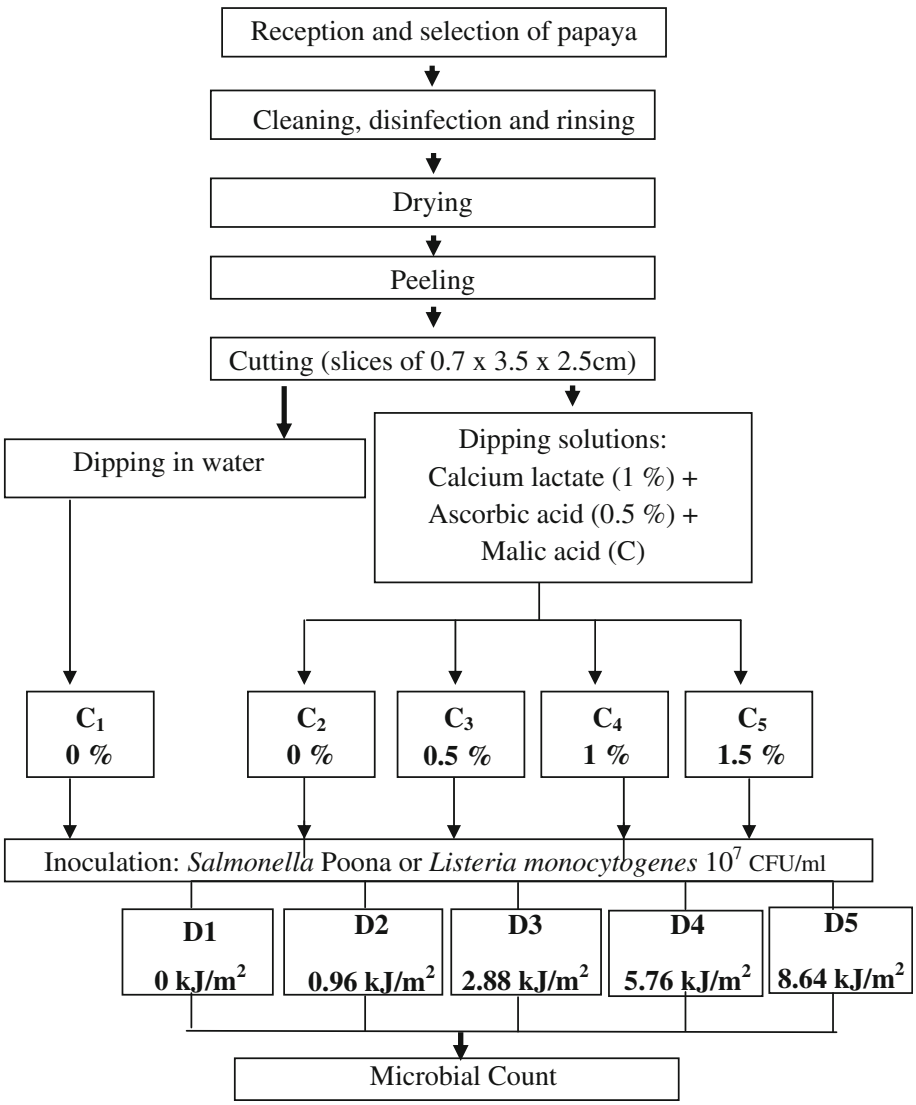

Fig. 1 Experimental design for evaluating the effect of different doses of UV-C light combined or not with malic acid on populations of *Salmonella* Poona or *Listeria monocytogenes* inoculated on fresh-cut "Maradol" papayas. Concentrations (%) of malic acid are indicated as C_1, C_2, C_3, C_4 and C_5; whereas UV-C light doses (KJ/m^2) are indicated as D_1, D_2, D_3, D_4 and D_5. Fifty combinations were randomly run by each microorganism based on a multi-level factorial design (5 UV-C light doses × 5 dipping conditions × 2 repetitions)

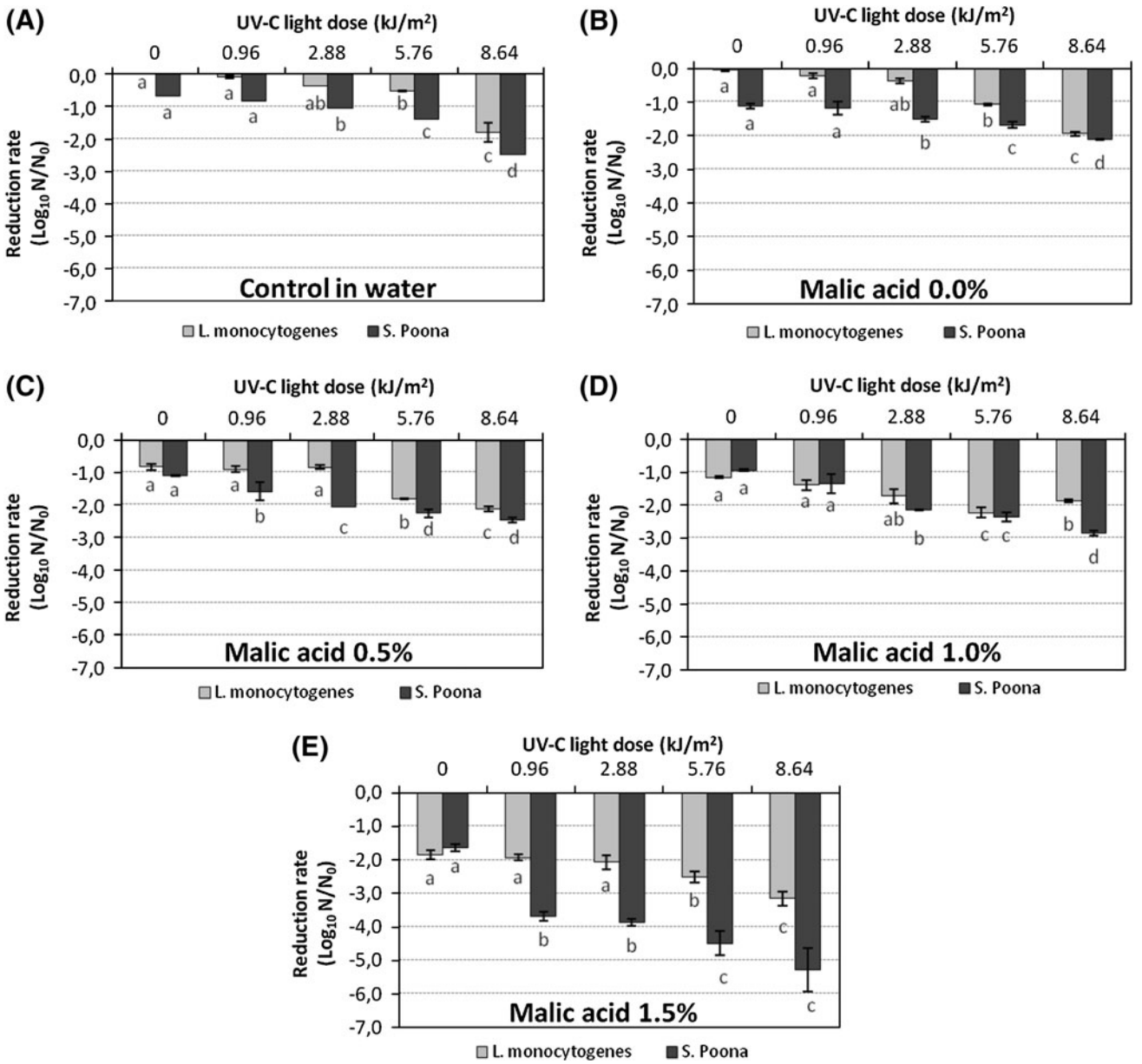

Fig. 2 Microbial reductions of *Salmonella* Poona and *Listeria monocytogenes* in fresh-cut "Maradol" papaya treated with distilled water (**A**), treated with stabilizing substances for color (ascorbic acid 0.5 %) and texture (calcium lactate 1 %) without malic acid (**B**), and treated with stabilizing substances plus malic acid (0.5, 1.0, 1.5 %) (**C**–**E**) applying different doses of UV-C light (0, 0.96, 2.88, 5.76, 8.64 kJ/m^2). *Bars* are the mean of two measurements in duplicate ($n = 4$). *Different lower-case letters* (*a*–*d*) indicate significant differences ($p < 0.05$) in microbial reductions by microorganism at different UV-C light doses

citric acid and 1 % ascorbic acid had no significant ($p > 0.05$) effect on *L. monocytogenes* populations. In contrast, Raybaudi-Massilia et al. (2009c) indicated that *L. monocytogenes* (about 0.5 Log_{10} CFU/g) and *Salmonella* Enteritidis (about 1.5 Log_{10} CFU/g) populations were significantly ($p < 0.05$) reduced on fresh-cut apples dipped into aqueous solutions containing calcium lactate (1 %), *N*-acetyl-L-cysteine (1 %) and glutathione (1 %) as quality stabilizing substances. DiPersio et al. (2003) reduced *Salmonella* population from 0.9 to 1.1 Log_{10} CFU/g on apple slices immersed in a citric acid solution at 0.21 % for 10 min. Differences found among authors could be attributed to the kind of fruit and strain of microorganism studied.

When malic acid was added to dipping solutions, a significant ($p < 0.05$) reduction of both pathogenic microorganisms was observed. The more effective concentration of malic acid was the higher one (1.5 %) (Fig. 2e). Lower concentrations (0.5 and 1.0 %) of this acid did not significantly ($p > 0.05$) affect the

population of *Salmonella* Poona (Fig. 2c, d; UV-C light doses = 0 kJ/m^2); whereas, populations of *L. monocytogenes* were significantly ($p < 0.05$) affected when concentrations of malic acid of 0.5 % were added (Fig. 2c–e; UV-C light doses 0 = kJ/m^2). These results demonstrated that *L. monocytogenes* populations were slightly more sensitive to malic acid than *Salmonella* Poona.

The antimicrobial effect of malic acid together with ascorbic acid and calcium lactate used in this experiment had a synergistic effect on the microbial inactivation due to the decrease of the pH in the cell cytoplasm. The malic acid, ascorbic acid and lactic acid (from dissociation of calcium lactate in aqueous solution Shelef 1994) could affect the cellular functions; because un-dissociated forms of weak organic acids may enter freely into microbial cell through water-filled channels formed by transmembrane proteins (porins) embedded into the lipid bilayer or through the peptidoglycan layer and, dissociate into charged anions and protons, due to higher pH of cell interior than exterior, thus acidifying the internal medium (by H^+ released) and affecting the cell signaling, glycolysis and active transport (Stratford and Eklund 2003). In addition, Raybaudi-Massilia et al. (2009d) indicated that the bactericidal action of organic acids may be influenced by the membrane structure of each microorganism. Nikaido (1996, 2003) has reported that the resistance mechanisms in Gram-negative bacteria are more complicated than those present in Gram-positive bacteria. The Gram-positive bacteria have a thick peptidoglycan layer and an inner lipid bilayer, but do not possess an outer membrane; therefore, weak organic acid may easily enter into cells. Gram-negative bacteria possess a thin peptidoglycan layer and inner and outer membranes. The hydrophobic nature of the outer membrane together with the lipopolysaccharides (located in the outer leaflet of the outer membrane) may block, delay or modulate the accessibility toward cell interior of hydrophilic molecules of low molecular mass such as monosaccharides, amino acids, nucleosides, weak organic acids, and alkali or alkaline ions (Nikaido 1996; Brul and Coote 1999).

Significant microbial reductions ($p < 0.05$) in fresh-cut papayas dipped in distilled water (Fig. 2a) and in quality stabilizing substances such as calcium lactate and ascorbic acid, but without malic acid (Fig. 2b) treated with UV-C light were observed. Doses of UV-C light from 2.88 and 5.76 kJ/m^2 were needed to significantly reduce populations of *Salmonella* Poona (1.5 Log_{10} CFU/g) and *L. monocytogenes* (1.0 Log_{10} CFU/g), respectively. In those samples treated with quality stabilizing substances plus malic acid, doses of UV-C light from 0.96 and 5.76 kJ/m^2 were enough for enhancing the microbial reductions of *Salmonella* Poona (from 1.5 to 3.8 Log_{10} CFU/g) and *L. monocytogenes* (from 2.0 to 2.5 Log_{10} CFU/g), respectively (Fig. 2c–e). These results demonstrate that populations of *Salmonella* Poona were more sensitive to the UV-C light than populations of *L. monocytogenes.* These results are according to Sharifi-Yazdi and Darghahi (Sharifi-Yazdi and Dargthahi 2006), who indicated that Gram-positive bacteria were more resistant to the UV light than Gram-negative, due probably to the many layers of peptidoglycan that the Gram-positive bacteria possess, forming thick and rigid cell walls, which may act like a shield against the penetration of the UV-C irradiation; while Gram-negative bacteria have only a single layer or a few layers of peptidoglycan. The germicidal effect of UV-C light has been acknowledged for more than a century. Rivera-Pastrana et al. (2007) and Gómez et al. (2010) have reported that even as UV-C radiation can be strongly absorbed by different cellular components, the most severe cell damage is due to reactions that occur when nucleic acids absorb UV-C light, which may cause mutations inhibiting or blocking the microbial cell replication and inducing cell death, if the threshold of crosslinked DNA molecules is exceeded.

Higher microbial reductions were observed when higher doses of UV-C light (8.64 kJ/m^2) and malic acid concentrations (1.5 %) were applied. Populations of *Salmonella* Poona and *L. monocytogenes* were reduced by more than 5 and 3 Log_{10} CFU/g, respectively, on fresh-cut papayas when that combination was used (Fig. 2e). In general, the combined treatments (UV-C light and malic acid) showed greater reductions of *Salmonella* Poona and *L. monocytogenes* populations than those observed in samples treated with distilled water alone or with quality stabilizing substances alone. These results indicate a synergistic effect between UV-C light and malic acid on the microbial inactivation, due probably to an induced stress in the cell by a decreasing of the surrounding and internal pH caused by the acid. Under these conditions, when UV-C light is applied; its genetic material is more exposed or sensible to the attack of irradiation, and a higher microbial inactivation is reached.

Just a few studies on the effect of UV-C light alone or in combination with organic acids to reduce or control pathogenic microorganisms in fresh-cut fruits have been reported. Yaun et al. (2004) applied UV-C light at 8.64 kJ/m^2 to surfaces of "Red Delicious"

apples inoculated with *E. coli* O157:H7, and reached to reduce 3.3 Log_{10} CFU/g, approximately; whereas, lower microbial reductions were observed on tomatoes inoculated with *Salmonella* spp. (2.19 Log_{10} CFU/g) using the same dose of UV-C light. The results obtained by these authors on the inactivation of *Salmonella* spp. are according to the ones reported by us, when solely UV-C light of the same dose had been applied, but without any organic acid added.

4 Conclusions

Significant reductions ($p \leq 0.05$) in the populations of *Salmonella* Poona or *L. monocytogenes* on fresh-cut "Maradol" papayas dipped in solutions containing quality stabilizing substances combined with different concentrations of malic acid and treated with different doses of UV-C light were observed. A synergistic effect against pathogenic microorganisms was found when a combination of UV-C light and malic acid was applied to fresh-cut "Maradol" papayas. Populations of *L. monocytogenes* were more resistant to the combination of UV-C light and malic acid than *Salmonella* Poona. In general, the results of this research showed that the use of malic acid in dipping solutions containing quality stabilizing substances in combination with UV-C light could be a good alternative to control pathogenic microorganisms in fresh-cut papayas.

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