Sanitation of Fresh Green Asparagus and Green Onions Inoculated with *Salmonella*

Miguel A. MARTÍNEZ-TÉLLEZ[1], Francisco J. RODRÍGUEZ-LEYVA[3], Ilce E. ESPINOZA-MEDINA[3], Irasema VARGAS-ARISPURO[2], Alfonso A. GARDEA[1], Gustavo A. GONZÁLEZ-AGUILAR[1] and J. Fernando AYALA-ZAVALA[1]

[1]*Coordinación de Tecnología de Alimentos de Origen Vegetal;* [2]*Coordinación de Ciencias de los Alimentos. Centro de Investigación en Alimentación y Desarrollo, A.C., Hermosillo, Sonora, México;* [3]*Unión Agrícola Regional de Productores de Hortalizas y Frutas de San Luís Río Colorado, Carretera a Riíto, Colonia La Mesa, San Luís Río Colorado, Sonora, México*

Abstract

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The absence of good agricultural and manufacturing practices in the production and postharvest handling of fresh produce, such as green asparagus or green onions increase the contamination risk by biological hazards like *Salmonella*. The objective of this work was to investigate the efficacy of chlorine (200 and 250 ppm), hydrogen peroxide (1.5% and 2%), and lactic acid (1.5% and 2%) sanitisers during different exposure times (40, 60, and 90 s) on the reduction of *Salmonella enterica subspecie enterica* serovar Typhimurium in inoculated fresh green asparagus and green onions. Washing with clean water only reduced < 1 $\log_{10}$ CFU/g in both vegetables. The most effective sanitiser evaluated for fresh green asparagus and green onions disinfection appeared to be 2% lactic acid reducing *Salmonella* growth close to 3 $\log_{10}$ CFU/g. Hydrogen peroxide was the least effective agent for *Salmonella* Typhimurium reduction. No effect was observed of the exposure time of inoculated product to sanitiser up to 90 seconds. These results confirm that lactic acid could be used as an alternative for fresh green asparagus and green onions sanitation.

Keywords: green asparagus; green onions; lactic acid; hydrogen peroxide; sodium hypochlorite; *Salmonella* Typhimurium

The consumption of fresh green asparagus (*Asparagus officinalis*) and green onions (*Allium fistulosum*) has been associated with lower risks of degenerative diseases (Hsing *et al.* 2002; Chin & Garrison 2008). Unfortunately, an increase has been observed in the frequency of the outbreaks of illnesses associated with the consumption of fresh produce (De-Roever 1998; Calvin 2003; CDC 2008; Cuite *et al.* 2009). The consumption of fresh green onions from Mexico was implicated in a Hepatitis A outbreak in USA (CDC 2003); on the other hand, fresh green asparagus is under the risk of pathogen contamination throughout its cultivation, harvest, packing, and distribution (Rodriguez-Leyva 2004). The application of good agricultural practices (GAP) in the field and good

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manufacturing practices (GMP) in the packinghouse, highlighting the sanitation practices, is the best option to warrant the safety of fresh produce (FDA 1998; Martínez-Téllez *et al.* 2005).

Salmonella is responsible for the most frequent foodborne diseases, being an important public health problem in almost all countries (D'Aoust 1997; Cummings *et al.* 2001; Calvin 2003; Greene *et al.* 2008). Several outbreaks of salmonelosis in the U.S.A. involved fresh fruits and vegetables (Cummings *et al.* 2001; Thunberg *et al.* 2002; Cuite *et al.* 2009). Fresh green asparagus and green onions are included in the list of highly perishable produce, which are susceptible to supporting the pathogens growth due to their high content of nutrients and relatively high pH values (5.5–7).

It is argued that the production, harvest, packing, storage, and transportation conditions of green asparagus and green onions represent a risk of *Salmonella* contamination (Beuchat 1995; Sapers *et al.* 2002; Parnell & Harris 2003). The studies on fresh green asparagus and green onions reported as possible sources of contamination the factors such as contaminated irrigation water, contaminated processing water, deficient hygienic practices, and inadequate cleaning and sanitising of the equipment coming in contact with the produce (Rodriguez-Leyva 2004). The produce disinfection procedures for the contamination control have been recommended for the reduction of microbiological hazards to thresholds not dangerous for the consumers (FDA 1998).

Washing is one of the most important steps during the fresh produce processing since it removes soil and microorganisms from the surface. However, not all washing methods and washing solutions are equally effective for all produce. Chemical sanitisers have been widely used in fresh fruit and vegetable disinfection. For many years, in the commercial processes, washing with chlorinated water has been the most utilised method to sanitise fresh produce. However, chlorinated water as disinfectant presents several disadvantages, the main being the inactivation of the active compound with organic matter; in this context, other sanitising agents have been tested, such as hydrogen peroxide and lactic acid (Wisniewski *et al.* 2000). Hydrogen peroxide is known as a very powerful oxidising agent that can be effective against a wide spectrum of microorganisms including bacteria, yeasts, molds, viruses, and spore-forming organisms (Cords & Dychdala 1993). Lactic acid (2-hydroxypropanoic acid) has shown antimicrobial activity against different microorganisms, its mode of action can be attributed to an acidification process causing depression of the internal pH of microbial cells by ionisation of the undissociated acid molecule, or disruption of the substrate transport by alteration of the cell membrane permeability. Food borne bacteria, like *Salmonella* spp., capable of causing human illness, cannot grow at pH lower than about 4.0, and for that reason the acidic pH of the edible portion of most fresh produce precludes their involvement as substrates for the proliferation of human pathogens. Regardless of the reported effectiveness of the above listed sanitisers, it has been observed that the sanitation efficacy depends on the concentration, exposure time, and treated product (Beuchat *et al.* 1998; Sapers *et al.* 2001; Sanz *et al.* 2002).

Therefore, this study was undertaken to investigate the efficacy of chlorine (200–250 ppm), hydrogen peroxide (1.5% and 2%) and lactic acid (1.5% and 2%) solutions on reducing *Salmonella* counts in inoculated fresh green asparagus and green onions exposed to sanitation for different time periods (40, 60, and 90 s) .

MATERIALS AND METHODS

Fresh produce preparation. Fresh green asparagus and green onions were obtained from packinghouses located in the San Luis Rio Colorado Valley of Sonora, Mexico. Fresh produce with bruises or other decay symptoms was excluded from the experiment, and stored at 4°C until needed. All samples were washed with sterile water, dried with a paper towel, and bunches of 6 to 8 units were formed before the experiment.

Bacterial strain. To minimise the growth on media of microorganisms naturally present on green asparagus and green onions, *Salmonella enterica* subspecie *enterica* serovar Typhimurium strain ATCC 14028 was grown in lactose broth (Difco Laboratories, Detroit, USA) supplemented with 50 µg/ml of nalidixic acid (Spectrum Inc., Gardena, USA). At the beginning of the study, a stock culture of ATCC 14028, the nalidixic resistant *Salmonella* Typhimurium, was prepared by adding sterile glycerol (Sigma, St. Louis, USA) to a final concentration of 16% to a 24 h old bacterial culture grown in lactose broth. Such culture was

dispensed into micro-tubes, mixed with glycerol and stored at – 55°C. For each experiment, the content of one micro-tube was thawed and used in the inoculum preparation.

Inoculum preparation. Prior to the experiment, a frozen 16% (w/v) glycerol stock culture was grown on lactose broth containing 50 µg/ml of nalidixic acid with the incubation at 35 ± 2°C for 24 hours. Then the microorganisms were cultured on xylose lysine desoxycolate agar containing 50 µg/ml of nalidixic acid at 35 ± 2°C for 24 h, and subsequently three loops of inoculum were transferred to one liter of lactose broth containing 50 µg/ml of nalidixic acid which was subsequently incubated at 35 ± 2°C for 18 h to obtain approximately 6.0 $\log_{10}$ CFU/ml of *Salmonella* Typhimurium.

Inoculation and sanitation procedure. Bunches of green asparagus and green onions were immersed for 1 min in a bacterial suspension containing approximately 6.0 $\log_{10}$ CFU/ml of *Salmonella* Typhimurium. The samples were drained and air-dried at ambient temperature (about 25°C), for 30 minutes. The sanitisers used were sodium hypochlorite (6%) (Cloralex, Santa Catarina, Mexico.), hydrogen peroxide (20%) (Sigma Aldrich, Inc. St. Louis, USA), and lactic acid (85%) (Sigma Aldrich, Inc. St. Louis, USA). The solutions were prepared and applied with sterile deionised water at 10°C. The concentrations of the chlorine solutions were 200 ppm and 250 ppm, pH 6.5 of hydrogen peroxide solution 1.5% and 2.0%, and of lactic acid solutions, pH 6.5, at 1.5% and 2.0%. The sanitation procedure was developed simulating a typical packinghouse washing process which included a spray application of the sanitisers solutions. After draining for 30 min, groups of 4 bunches of green onions and green asparagus were selected for each treatment application; the bunches were placed on a sterile wire screen and were sprayed at 10°C for 40, 60, and 90 s either with sterile distilled water or with the sanitisers mentioned above.

Salmonella counts. After the treatment, the bunches were drained for 20 min and individually placed in sterile plastic bags to be crushed, and then 10 g of each sample were transferred to a new bag containing 90 ml of sterile 0.1% peptone solution (Becton Dickinson, Spark, USA) followed by vigorous agitation for 1 minute. The inoculated bunches not subjected to any sanitation treatment or water were analysed for *Salmonella*, as well as those used for the initial counts to determine the sanitisers effectiveness. The number of viable cells was determined by serial 0.1% peptone water dilutions (1:10). To quantify *Salmonella*, duplicates of 1 ml of each dilution were added to approximately 15 ml of xylose lysine desoxycolate agar (Difco Lab., Detroit, USA) containing 50 µg/ml of nalidixic acid (Spectrum Inc., Gardena, USA). The plates were incubated at 35 ± 2°C for 24 h, and the typical *Salmonella* colonies were counted.

Statistical analysis. A completely randomised experimental design with 3 replicates was used with a 3 × 3 factorial arrangement of the treatments, the sanitiser concentration and exposure time as factors. Analysis of variance (ANOVA) was performed using the NCSS software (Hintze 2001). Duncan's test for mean comparison was used with the same program. The significance was defined as $P < 0.05$.

RESULTS AND DISCUSSION

The initial analysis of the fresh green asparagus and green onions that were not inoculated revealed the absence of *Salmonella*. Green onions washed with sterile water only reduced 0.65 log CFU/g, these results are in agreement with previous reports by Yu *et al.* 2001, who found that water-washed strawberries inoculated with *Escherichia coli* reduced 0.75 log CFU/g. Similarly, Sapers *et al.* (2002) working with fresh cut melon observed a reduction of 0.45 log CFU/g.

Efficacy of chlorine sanitation

Figure 1 shows the effect of chlorine sanitation on the inhibition of inoculated *Salmonella* in green onions (I) and asparagus (II) at different exposure times. A significant effect ($P < 0.05$) of the chlorine concentration on the efficacy of the sanitation process was observed. Chlorine sanitation showed a better efficacy at a higher concentration (inhibition of *Salmonella* at 1.36–1.74 $\log_{10}$ CFU/g), however, no significant difference ($P > 0.05$) between 200 ppm and 250 ppm Cl_2, was observed. Chlorine showed a more effective *Salmonella* inhibition on the inoculated fresh green onions as compared to asparagus spears, nevertheless, no significant effect of the exposure time was observed.

Different studies reported the efficacy of different extents of chlorine sanitation on fruit inoculated with different pathogens. Sapers *et al.* (2002)

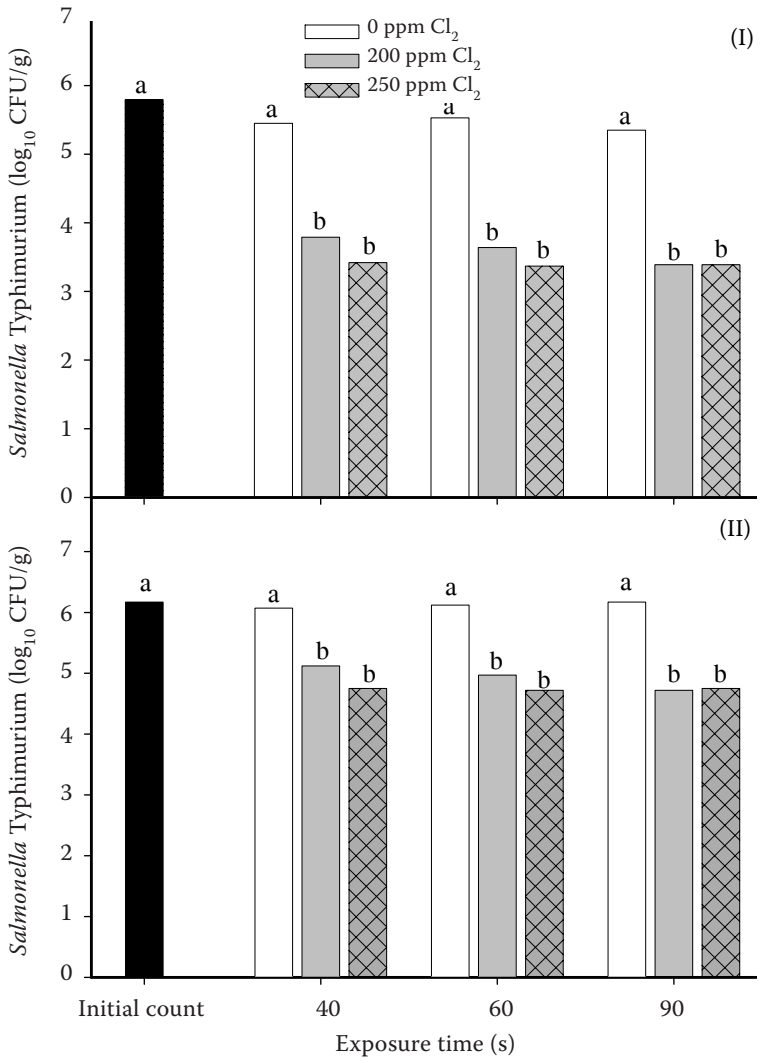

Figure 1. Effect of chlorine sanitation on the reduction of inoculated *Salmonella* in green onions (I) and asparagus (II) at different exposure times. Different letters amongst bars of each exposure time represent significant differences ($P < 0.05$).

found that chlorine sanitation (200–250 ppm Cl_2) inhibited 1.74 $\log_{10}$ CFU of *E. coli* on inoculated apples. On cantaloupes, an inhibition was reported of 1.86 $\log_{10}$ CFU of Gram negative bacteria (Ukuku & Fett 2002). Moreover, in other study where chlorine (> 200 ppm) was used to reduce *Salmonella* and *E. coli* O157:H7, the reduction was of 2.3 $\log_{10}$ CFU/cm^2 in apples, tomatoes, and lettuces (Beuchat *et al.* 1998). A more effective sanitation process was achieved using chlorine to reduce *Salmonella* on inoculated apples, with an inhibition of 3.2 $\log_{10}$ CFU/g (Parnell & Harris 2003).

Although chlorine is an effective sanitiser for fruits and vegetables, its antimicrobial activity is diminished by organic matter in the sanitation solution (Beuchat *et al.* 1998). For example, it has been demonstrated that maintaining the desired level of free available chlorine in the washing solution in the processing plant is difficult due to the accumulation of organic matter (Garg *et al.* 1990). Since the sanitation solution is often recycled, a high organic matter content reduces the activity of chlorine (Brackett 1992) and increases the likelihood of fresh produce contamination.

Efficacy of hydrogen peroxide sanitation

The effect of hydrogen peroxide sanitation on the inhibition of inoculated *Salmonella* in green onions (I) and asparagus (II) at different exposure times is showed in Figure 2. A significant effect ($P < 0.05$) of H_2O_2 concentration on the efficacy of the sanitation process was observed. Hydrogen peroxide sanitation showed a better efficacy at a higher concentration (inhibition of *Salmonella* at 1–1.43 $\log_{10}$ CFU/g), on the other hand, no significant differences ($P > 0.05$) were observed between 1.5% and 2% H_2O_2. No significant differences

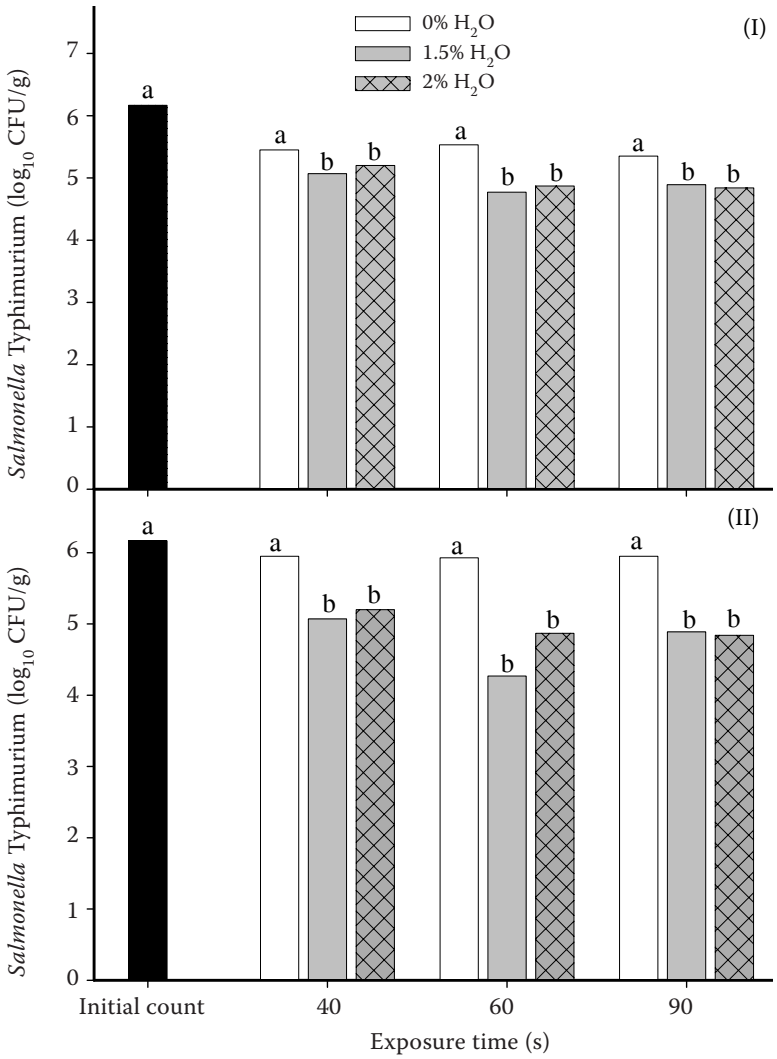

Figure 2. Effect of hydrogen peroxide sanitation on the reduction of inoculated *Salmonella* in green onions (I) and asparagus (II) at different exposure times. Different letters amongst bars of each exposure time represent significant differences ($P < 0.05$)

($P > 0.05$) were observed between the exposure times and between fresh produce. Comparing the efficacy of hydrogen peroxide sanitation with that of chlorine, it was observed that chlorine showed a more effective *Salmonella* inhibition with inoculated fresh green onions and asparagus spears.

Reports have been published on the use of hydrogen peroxide as fresh produce sanitiser. Yu *et al.* (2001) found that utilising hydrogen peroxide at 1% achieved reductions of 1.2–1.4 log CFU/g of strawberries inoculated with *E. coli,* while 3% hydrogen peroxide achieved reductions in the order of 2.18 log_{10} CFU/g. Sapers *et al.* (2001, 2002) reported that 5% of H_2O_2 inhibited 2.34 log_{10} CFU/g of *E. coli* inoculated on apples, however, this treatment was applied in conjunction with high temperatures (50–70°C), which could affect the freshness of the sanitised produce .

Hydrogen peroxide is known to be a very powerful oxidising agent that is in general effective against a wide spectrum of microorganisms including bacteria, yeasts, molds, viruses, and spore-forming organisms (Cords & Dychdala 1993). However, the concentrations of hydrogen peroxide to elicit significant microbial reduction ranges were over the target concentrations of hydrogen peroxide (50–100 ppm) in the commercial mixtures (Beuchat & Ryu 1997; Park & Beuchat 1999; Taormina & Beuchat 1999). Other research showed that hydrogen peroxide at 10 000–50 000 ppm, applied alone or in combination with other organic acids could only effect ≤ 5 log reductions of microbial contamination of fruits and vegetables (Beuchat & Ryu 1997; Park & Beuchat 1999).

Hydrogen peroxide is a very reactive compound whose efficacy as sanitiser may be affected by inactivation with the contacted surfaces, including the treated fresh produce (Azanza 2004). The concentrations of hydrogen peroxide on tomatoes, broccoli, and potatoes washed in a solution con-

taining 59 ppm hydrogen peroxide, with moderate agitation, 5 min contact time, 21–24°C, were not significantly different before and after the treatment ($P > 0.01$) (Azanza 2004). These results were interpreted as non-reactivity of the active agent with the components of fruit and vegetable samples analysed. However, about 37% drop in ascorbic acid (oxidised form of Vitamin C) content of tomatoes was detected, with an equivalent increase in dehydroascorbic acid (reduced form of Vitamin C) content, using the same treatment (Azanza 2004). It has been shown that hydrogen peroxide is generally used in much higher concentrations ranging from > 50,000 ppm to 350 000 pm when used either as vapour or for immersion treatments. Lower concentrations of hydrogen peroxide (≤ 3 ppm) were also reported but these were restricted only to the vapour treatment. The published information also indicated that hydrogen peroxide causes browning, bleaching, and blistering of sanitised food materials (Azanza 2004).

Efficacy of lactic acid sanitation

Figure 3 shows the effect of lactic acid sanitation on the inhibition of inoculated *Salmonella* in green onions (I) and asparagus (II) at different exposure times. A significant effect ($P < 0.05$) of lactic acid concentration and the treated fresh product on the efficacy of the sanitation process was observed. Lactic acid sanitation showed better efficacy at the highest concentration, 2% (inhibition of *Salmonella* at 2.9 $\log_{10}$ CFU/g), however, significant differences ($P < 0.05$) between the sanitised fresh produce were observed. Lactic acid showed a more effective *Salmonella* inhibition on inoculated fresh asparagus spears as compared to green onions, nevertheless, no significant effect ($P > 0.05$) of the exposure time was observed. Comparing the efficacy of lactic acid sanitation with that of chlorine sanitation, it was observed that lactic acid showed a more effective *Salmonella* inhibition on inoculated fresh asparagus spears.

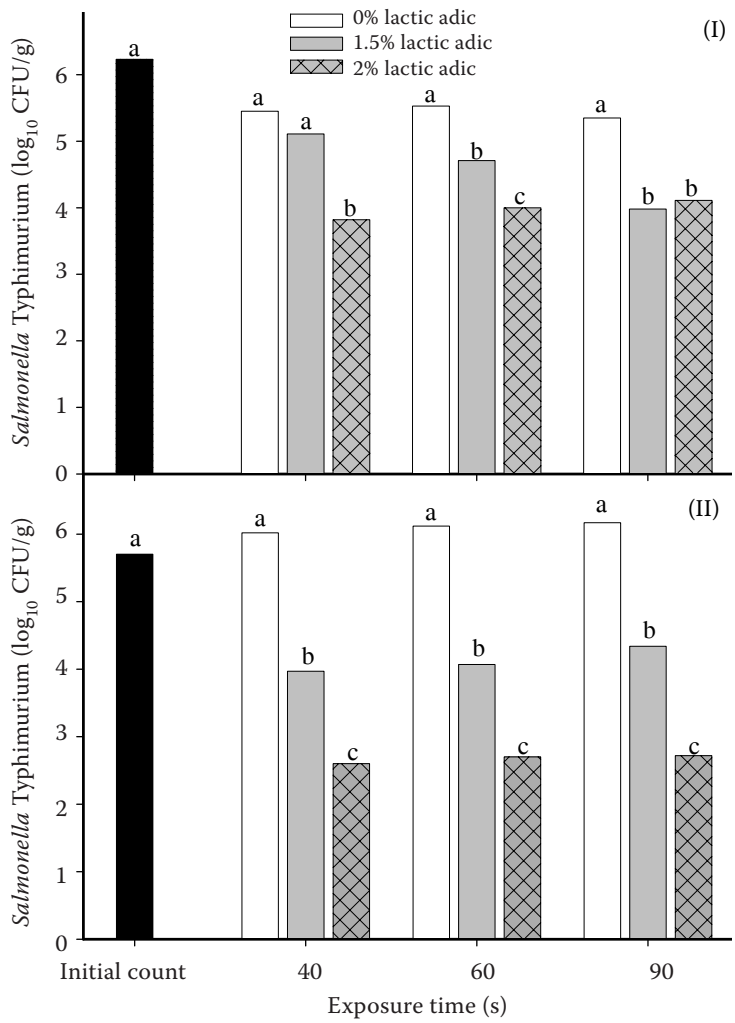

Figure 3. Effect of lactic acid sanitation on the reduction of inoculated *Salmonella* in green onions (I) and asparagus (II) at different exposure times. Different letters amongst bars of each exposure time represent significant differences ($P < 0.05$)

The use of 2% lactic acid on asparagus reduced 2.9 $\log_{10}$ CFU/g, in agreement with the results found previously (Ibarra-Sánchez *et al.* 2004) when using the same concentration of lactic acid to reduce *Salmonella* Typhimurium in apples. 2% lactic acid also reduced 2.9 $\log_{10}$ CFU/g in *Salmonella* populations from tomatoes. 3% lactic acid caused a 2.18 $\log_{10}$ reduction in strawberries inoculated with *E. coli* O157:H7 (Yu *et al.* 2001). However, increasing the concentration up to 5% was required to reduce 2.1 $\log_{10}$ CFU/g in apples inoculated with *Salmonella enterica* (Parnell & Harris 2003). Total and fecal coliforms were reduced about 2 and 1 log/g, respectively, on mixed salad vegetables treated with 1% lactic acid (Torriani *et al.* 1997). In the same study, the treatment of the mixed vegetables with a 3% sterile permeate from a culture of *Lactobacillus casei* reduced the total mesophilic count about 5 log/g and prevented the growth of coliforms, enterococci, and *Aeromonas hydrophila* after 6 days at 8°C. The solution of 1.5% lactic acid with 1.5% hydrogen peroxide was successfully used in eliminating *E. coli* O157:H7 and *Salmonella* on some fruits and vegetables without affecting the sensory and qualitative characteristics of the produce (Singh *et al.* 2002; Venkitanarayanan *et al.* 2002).

Lactic acid is used as a flavour enhancer, antimicrobial agent, and pH control agent, regarded as safe (GRAS) for the use in food products (Eswaranandam *et al.* 2006). It has been reported that the antimicrobial activity of this organic acid can be enhanced by combining it with other food preservatives or by increasing the temperature at washing. It has been reported that the immersion of some fruits in a 1.5% solution of lactic acid with 1% or 1.5% hydrogen peroxide eliminated the attached populations of *E. coli* O157:H7, *Salmonella enteritidis*, and *Listeria monocytogenes* (Venkitanarayanan *et al.* 2002). There appears to be little or no additional benefit from adding hydrogen peroxide to the lactic acid solution over that obtained with lactic acid alone. No published information on the effect of the exposure time on the sanitiser efficacy was found.

CONCLUSIONS

The most effective sanitiser evaluated for fresh asparagus and green onions disinfection was 2% lactic acid followed by 250 ppm chlorine. Hydrogen peroxide for *Salmonella* Typhimurium reduction. No effect of the exposure time of the inoculated produce to sanitiser was observed up to 90 s. These results confirm that lactic acid could be used as an alternative to chlorine for fresh asparagus and green onions sanitation.

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Corresponding author:

Dr. Miguel A. Martínez-Téllez, Centro de Investigación en Alimentación y Desarrollo, A.C., Coordinación de Tecnología de Alimentos de Origen Vegetal, Carretera a la Victoria km 0.6, La Victoria, Hermosillo, Sonora, 83000 México
tel.: + 152 662 284 2400, fax: + 52 662 280 0422, e-mail: norawa@ciad.mx