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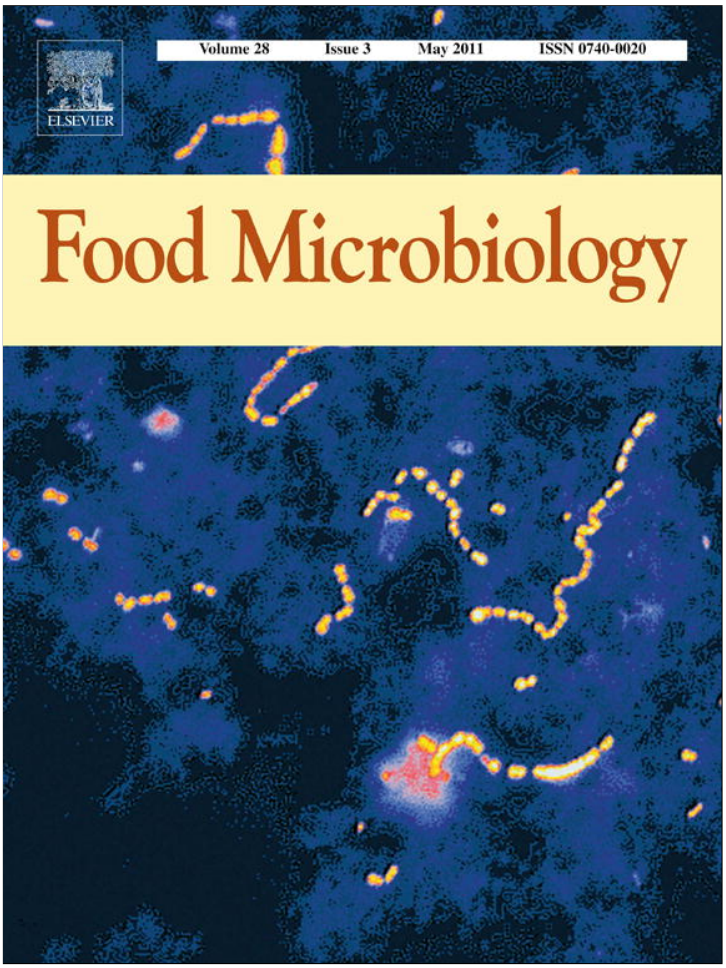

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Combination treatment of alkaline electrolyzed water and citric acid with mild heat to ensure microbial safety, shelf-life and sensory quality of shredded carrots

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ABSTRACT

The objective of this study was to determine the synergistic effect of alkaline electrolyzed water and citric acid with mild heat against background and pathogenic microorganisms on carrots. Shredded carrots were inoculated with approximately 6–7 log CFU/g of *Escherichia coli* O157:H7 (932, and 933) and *Listeria monocytogenes* (ATCC 19116, and 19111) and then dip treated with alkaline electrolyzed water (AlEW), acidic electrolyzed water (AcEW), 100 ppm sodium hypochlorite (NaOCl), deionized water (DaIW), or 1% citric acid (CA) alone or with combinations of AlEW and 1% CA (AlEW + CA). The populations of spoilage bacteria on the carrots were investigated after various exposure times (1, 3, and 5 min) and treatment at different dipping temperatures (1, 20, 40, and 50 °C) and then optimal condition (3 min at 50 °C) was applied against foodborne pathogens on the carrots. When compared to the untreated control, treatment AcEW most effectively reduced the numbers of total bacteria, yeast and fungi, followed by AlEW and 100 ppm NaOCl. Exposure to all treatments for 3 min significantly reduced the numbers of total bacteria, yeast and fungi on the carrots. As the dipping temperature increased from 1 °C to 50 °C, the reductions of total bacteria, yeast and fungi increased significantly from 0.22 to 2.67 log CFU/g during the wash treatment ($p \leq 0.05$). The combined 1% citric acid and AlEW treatment at 50 °C showed a reduction of the total bacterial count and the yeast and fungi of around 3.7 log CFU/g, as well as effective reduction of *L. monocytogenes* (3.97 log CFU/g), and *E. Coli* O157:H7 (4 log CFU/g). Combinations of alkaline electrolyzed water and citric acid better maintained the sensory and microbial quality of the fresh-cut carrots and enhanced the overall shelf-life of the produce.

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1. Introduction

The consumption of fresh fruits and vegetables is increasing rapidly in Korea as well as throughout the world due to their health effects (Huxley et al., 2004). In addition, the demand for ready-to-use shredded and sliced carrots has become widespread. Accordingly, the minimally processed fresh-cut produce industry has been growing rapidly over the past decade, stimulated by strong consumer demand for ready-to-eat produce that is convenient and nutritious (IFPA, 2000). However, the frequency of outbreaks of foodborne illnesses associated with consumption of fresh fruits and vegetables has increased in recent years, in part due to the increased demand for fresh produce (Nguyen-the and Carlin, 1994; Beuchat, 1996; Roever, 1998; Francis et al., 1999). This is because the processing steps (peeling and cutting) enhance the susceptibility of these products to microbial growth (Tournas, 2005). Indeed, various bacterial pathogens have been reported to survive and grow on fruits and vegetables (Nguyen-the and Carlin, 1994).

Washing is one of the most important steps during the processing of produce since it removes soil and microorganisms from the surface. The use of effective sanitizing agents during the washing of produce is necessary to ensure product safety. In minimally processed vegetables, such as shredded carrot, chlorine solutions have been widely employed by the industry for sanitization purposes. However, reduced microbiological efficiency coupled with sensorial changes and the eventual formation of carcinogenic chlorinated compounds have demonstrated the need for alternative decontamination methodologies. Sanitizing treatments reported to reduce bacteria on carrots include rinsing in lemon juice and lemon juice vinegar (Sengun and Karapinar, 2004, 2005) and anolyte water and chlorinated water (Workneh et al., 2003). Chlorine dioxide, ozone, and thyme essential oil have also been used to decontaminate carrots (Singh et al., 2002). Moreover, a 3 log decrease in microbial load was observed in response to heat treatment (Alegria et al., 2009, 2010). Other methods of sterilization that have been

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tested include acidified sodium chlorite (Ruiz-Cruz et al., 2006, 2007), peroxyacetic acid (Vandekinderen et al., 2009), and irradiation (Chaudry et al., 2004) treatment. Warm water (Klaiber et al., 2005) and electrolyzed water (Izumi, 1999) have also been found to decrease the populations of bacteria on carrots.

The combined use of several disinfectant agents has been widely reported in the last few years (Beltrán et al., 2005; Ukuku et al., 2005; Uyttendaele et al., 2004). Combinations of lactic acid, chlorinated water, thyme essential oil solution, sodium lactate, citric acid, hydrogen peroxide, ozone and peroxyacetic acid have previously been tested and combinations of chemical disinfectants have generally been found to maintain better sensory and microbial quality of the product. Additionally, a novel produce-washing procedure using a combination of alkaline electrolyzed water (AlEW), AcEW and mild heat induced a significant bactericidal effect when compared with treatment at ambient temperature (Koseki and Isobe, 2007). Recently, combinations of alkaline electrolyzed water and citric acid showed a strong synergistic antimicrobial effect that reduced background flora and foodborne pathogens on fresh-cut produce and cereal grains (Park et al., 2004, 2009; Rahman et al., 2010).

Minimally processed (MP) carrots constitute one of the major minimally processed vegetables (MPV). The main problems that limit the shelf-life of MP carrots are white blush discolouration caused by tissue dehydration and microbial spoilage (Emmambux and Minnaar, 2003). Therefore, treatments that can inactivate the natural microflora while keeping the tissue hydrated may prolong their shelf-life. Thus the objective of this study was to investigate the synergistic effect of alkaline electrolyzed water and citric acid with mild heat on the microbiological quality of shredded carrots measured immediately after treatment, as well as to study the shelf-life and sensory attributes during subsequent storage at 4 and 25 °C.

2. Materials and methods

2.1. Preparation of inocula

The two strains of *Listeria monocytogenes* (ATCC 19116 and ATCC 19111) and *Escherichia coli* O157:H7 (932 and 933) used in this experiment were obtained from Korean National Institute of Health (Seoul, Korea) and Department of Food Science, University of Georgia (Griffin, GA, USA), respectively. Stock cultures were maintained on tryptic soy agar (TSA) (BD Diagnostics, Franklin Lakes, NJ, USA) slants at 4 °C. Prior to use, all strains were separately grown in tryptic soy broth (TSB) (BD Diagnostics) at 37 °C with two consecutive transfers after 24 h periods for a total of 48 h of incubation. All working cultures (approximately 10^9 CFU/mL) grown in TSB were separately centrifuged at 4000×*g* for 15 min at 4 °C and the supernatants were discarded. The cell pellets were washed twice with 0.1% peptone water (pH 7.1) and resuspended in 10 mL of the same solution to obtain a final cell concentration of 10^9 CFU/mL. After this, two strains of *E. coli* O157:H7 and *L. monocytogenes* were combined in a cocktail with approximately equal numbers in the final population. These culture cocktails were used in subsequent experiments. The bacterial population in each cocktail culture was confirmed by plating 0.1 mL portions of appropriately diluted culture on tryptic soy agar (TSA) (Difco Laboratories) plates and incubating the plates at 35 °C for 24 h.

2.2. Preparation of carrot samples

Fresh carrot (*Daucus carota* L.) roots were purchased from a local wholesale market in Chuncheon, Korea and then transported to the laboratory and used within 24 h following storage at 4 °C. The carrot root tips and leaf ends were removed and roots with visible damage were discarded. Carrots were washed with tap water to remove the soil and then hand peeled and shredded into 3 × 3 cm pieces (2–5 mm wide) using a knife that had been dipped into 70% (v/v) alcohol and flamed before use. Aliquots with a weight of 10.0 ± 0.3 g were then used for subsequent analyses.

2.3. Inoculation of carrot samples

To destroy the background microflora, 10 g of carrots were placed on a sterile perforated tray and treated with UV light (Philips, TUV 15W) in a UV cabinet (Entkeimungsschrank, 220 V, Ernst Schuttjun Laborgerotebau, 3400, Gottingen) for 50 min (25 min for each side) (Sengun and Karapinar, 2005; Singh et al., 2002). After applying this treatment, the naturally existing bacterial population was reduced to an undetectable level (with 10 CFU/g detection limit). Accordingly, pieces of carrots (10 g each) were placed into sterile bags and then sprinkle inoculated with the mixed inocula (1 mL from each) of *E. coli* O157:H7 and *L. monocytogenes* to obtain an initial level of 10^7 log CFU/g. The samples were then shaken vigorously for 3 min so that the inoculum would be evenly distributed. To allow attachment of bacteria, inoculated carrots were air-dried in a laminar flow hood for 1 h at 22 ± 2 °C and then stored in a refrigerator at 4 °C for 24 h before exposure to various sanitizing treatments.

2.4. Preparation of sanitizers

Electrolyzed water (EW) was produced from 0.1% NaCl solution using a flow-type electrolysis (A2-1000, Korean E & S Fist Inc., Seoul, Korea) set at 16 A. When a stable amperage was reached after 15 min, AcEW (pH of 2.4–2.6, ORP of 1000 mV–1100 mV, and an available chlorine concentration of 50–60 mg/L) and AlEW (pH of 11–11.2 and ORP of −830 mV to −850 mV) were collected from the anode compartment and the cathode respectively, separated by a membrane. The sodium hypochlorite solution (NaOCl: pH 9.8, 100 mg/L available chlorine) was prepared with the addition of 0.1 g of NaOCl (DC Chemical Co., Seoul, Korea) in 1 L of sterile distilled water. The crystalline citric acid (CA) (Yakuri Pure Chemicals Co., Kyoto, Japan) was dissolved in 1 L of sterile distilled water to give a final concentration of 1% CA solution (w/v). Sterile deionized water (DIW) was used as a control. The pH, ORP and available chlorine concentration of treatment solutions were measured immediately before treatment with a dual-scale pH meter (Accumet model 15, Fisher Scientific Co., Fair Lawn, NJ) with pH and ORP electrodes. The residual chlorine was determined by a colorimetric method using a digital chlorine test kit (RC-3F, Kasahara Chemical Instruments Co., Saitama, Japan). The detection limit is 0–300 mg/L.

2.5. Sanitization treatment

The following sanitizer treatments were evaluated for their efficacy in killing or reducing *E. coli* O157:H7, *L. monocytogenes* and background flora on shredded carrots. Inoculated and uninoculated carrot samples (10 g) were placed in sterile containers and immersed in each sanitizing agent. The carrot to solution ratio in all treatments was 1:10 (w/v). Both DIW washed and unwashed carrot samples were used as controls. To evaluate the effect of dipping time on the reduction of microorganisms, each piece (10 g) of uninoculated samples was dipped for 3, 5, and 10 min and the samples were then immersed at 1, 20, 40, and 50 °C to evaluate the effect of temperature. The following dipping solutions were used either alone or in combination with AlEW as dipping agents: DIW, 100 ppm NaOCl, electrolyzed water (AcEW and AlEW) and 1% CA

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(Fisher Scientific Co., Norcross, GA, USA). After dipping each carrot, the moisture on each piece was drained using sterile gauze. All individual experiments were replicated three times.

2.6. Microbiological analyses

Following treatments, all samples (each of 10 g) were aseptically excised and immediately placed in a stomacher bag (Nasco Whirl-Pak, Janesville, WI, USA) containing 90 mL of buffered peptone water (BPW; Difco, Sparks, Md., USA) and then homogenized for 2 min using a Seward stomacher (400 Circulator, Seward, London, UK).

Following homogenization, 1-mL aliquots of the sample were serially diluted in 9 mL of sterile BPW and 0.1 mL of sample or diluent was spread-plated onto each selective medium (in this case, the limit of detection was 100 CFU/g, which is equivalent to 2 log CFU/g). In the case of 1 mL placed on the plates, the limit of detection was 1 log CFU/g. The selective media, Sorbitol MacConkey agar (Difco) and Oxford Agar Base (Difco), were used for enumeration of *E. coli* O157:H7 and *L. monocytogenes*, respectively. For uninoculated carrot samples, the mixtures were plated onto plate count agar (Difco) for the total counts and potato dextrose agar (Difco with 10% tartaric acid solution) to enumerate the yeast and fungi. All plates were incubated at 37 °C for 24 h, except for yeast and fungi plates, which were incubated at 25 °C for 3–5 days. The results were expressed as the log CFU/g.

2.7. Shelf-life study

For the shelf-life study, uninoculated carrot (untreated and treated) samples were packaged using Ziploc® vegetable bags (Howard et al., 1999) and then marked carefully before storage at different temperatures (4, and 25 °C). During storage, sampling was conducted at 2-day intervals for 4 °C, while samples were only collected at 3 to 12-h intervals for samples stored at 25 °C. At each sampling interval, 90 mL of 0.1% sterile peptone water were poured into the Stomacher bag (Nasco Whirl-Pak, Janesville, WI, USA) containing the sample. The samples were then homogenized for 2 min using a Seward stomacher (400 Circulator, Seward, London, UK), after which 0.1 mL aliquots of the appropriate dilution were spread onto the surface of the duplicate plates of plate count agar (Difco) for the total counts and potato dextrose agar (Difco with 10% tartaric acid solution) for enumeration of the yeast and fungi. The bacterial plates were then incubated at 37 °C for 24 h, while the yeast and fungi plates were incubated at 25 °C for 3–5 days and expressed as log CFU/g. Each experiment was replicated three times, and the means of microbial populations (log CFU/g) from different storage temperatures were calculated. The end of the shelf-life arrived when the population of a group of microorganisms reached an unacceptable level or when the sensory panel rejected the samples.

2.8. Evaluation of the sensory quality during the shelf-life

Sensory evaluation of shredded carrots (treated and untreated) was conducted for 3-day intervals during storage (15 days) at 4 °C. A panel of five trained judges (members of our department) was then assigned to distinguish the following sensory attributes: color, firmness, fresh-like aroma and general acceptance. The attributes were scored using a 5-point numeric rating scale (Alegria et al., 2009) as follows:

Color: 1 = very light orange; 2 = light orange; 3 = orange; 4 = dark orange; 5 = very dark orange, where 3 is an anchor corresponding to the initial carrot fresh appearance immediately after cutting;
Firmness: 1 = very firm; 2 = firm; 3 = moderate; 4 = soft; and 5 = very soft, where 1 is an anchor corresponding to the perception of the fresh-cut carrot immediately after cutting;
Fresh-like aroma: 1 = very intense; 2 = intense; 3 = moderate; 4 = low; and 5 = absent, where 1 is an anchor corresponding to the perception of the carrots aroma immediately after cutting. Panelists were also asked to identify the presence of any off-odors in the comments section;
General acceptance: 1 = excellent freshly cut; 2 = good; 3 = limit of marketability; 4 = fair limit of usability; and 5 = poor unusable.

Panelists were also asked to provide any relevant comments.

The mean scores of the attribute intensity and acceptance were calculated and the end of the shelf-life from the sensory point of view was reached when at least one of the mean scores was above the middle point of the respective scale. The applied cut-off for the general acceptance was fixed at 3 (limit of marketability), and scores above 3 indicated unacceptable samples.

2.9. Statistical analyses

All analyses were conducted in duplicates with 3 replicates of each experiment. Data were expressed as the means ± standard errors. The results were analyzed using the SPSS statistical package (SPSS Inc., Chicago, IL). An analysis of variance (ANOVA) was used to evaluate the treatment, dipping time, and temperature as fixed effects. Tukey's multiple range tests were used to determine the significant difference at $p \leq 0.05$.

3. Results and discussion

The properties (pH, ORP, and residual chlorine) of treatment solutions (AlEW, AcEW, NaOCl, 1% CA, AlEW + 1% CA and DIW) used in this study are presented in Table 1.The washing effect of DIW, NaOCl, AlEW and AcEW in shredded carrots against background flora was observed in this study. When compared to unwashed controls, different wash solutions reduced the total bacteria by 0.46–2.25 log CFU/g (Fig. 1A) and the yeast and fungi by 0.30–2.25 log CFU/g (Fig. 1B) after different dipping times (1, 3 and 5 min). For all dipping times, DIW treatment reduced the populations by less than 0.9 log CFU/g, while AcEW treatment showed the greatest reduction of 2.25 log CFU/g for the total bacteria and yeast and fungi count, respectively compared to the untreated control. Three and 5 min dipping time from each treatment except DIW showed better effect than 1 min on the reduction of total

Table 1
Physicochemical properties of washing solutions.[a]

Washing solutions	pH	ORP (mV)	ACC[b] (mg/L)
AlEW[c]	11.3 ± 0.2	−810 ± 15	Not measured
AcEW[d]	2.54 ± 0.3	1100 ± 20	50 ± 2.2
NaOCl[e]	9.8 ± 0.2	690 ± 25	100 ± 4.5
1% CA[f]	2.23 ± 0.04	970 ± 10	Not measured
AlEW + 1% CA[g]	2.48 ± 0.02	550 ± 15	Not measured
DIW[h]	7.0 ± 0.1	412 ± 18	0.3 ± 0.1

[a] Values are mean ± standard deviation, $n = 3$.
[b] Available chlorine concentration.
[c] Alkaline electrolyzed water.
[d] Acidic electrolyzed water.
[e] Sodium hypochlorite solution.
[f] Citric acid (1%).
[g] Alkaline electrolyzed water + 1% Citric acid.
[h] Deionized water.

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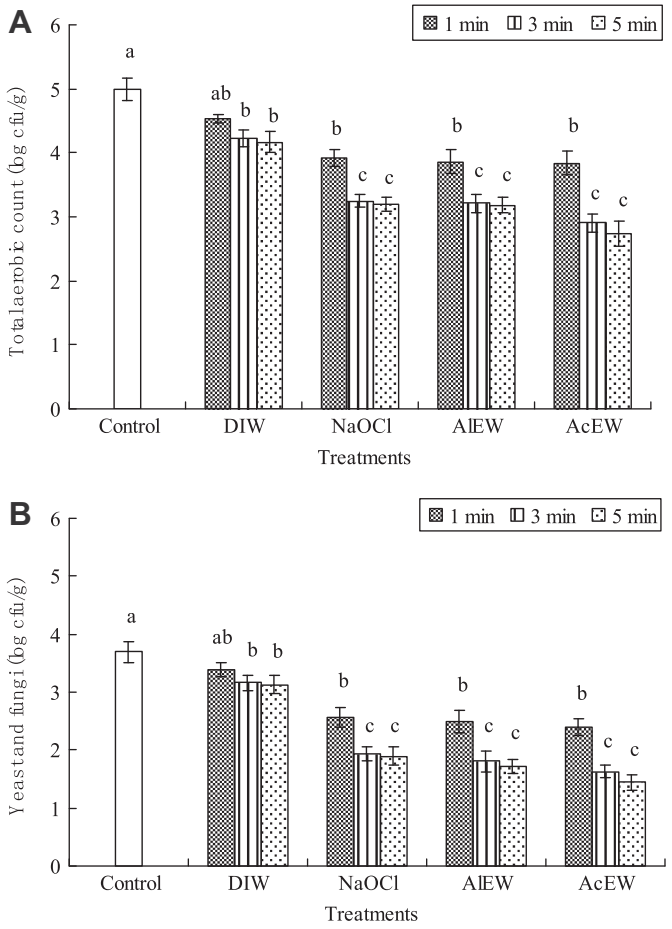

Fig. 1. Inactivation effect of electrolyzed water on the total counts (A), and yeast and fungi (B) in carrots by different dipping times. Vertical bars represent mean ± standard error of mean, $n = 6$. Bars labeled with different letters in the same treatment indicate a significant difference ($p \leq 0.05$). (Control: not treated; DIW: deionized water; NaOCl: 100 mg/L sodium hypochlorite; AlEW: alkaline electrolyzed water; AcEW: acidic electrolyzed water).

counts on carrot (Fig. 1A). Similar results were observed in the reduction of yeast and fungi on carrot (Fig. 1B). From these results, there was no any remarkable difference ($p \geq 0.05$) found in microbial reduction between 3 and 5 min dipping. Also 5 min dipping resulted in changing color (Visual estimation) of shredded carrots. According to these results, 3 min exposure would be the most appropriate to minimize the quality deterioration (color), thus it was chosen for following treatments. Washing with sanitizers also significantly ($p \leq 0.05$) reduced the background flora when compared to the unwashed control. The relative bacterial reduction potency occurred in the order AcEW > AlEW > NaOCl > DIW. The sanitizing effect of electrolyzed water against total microflora on fresh-cut vegetables was first reported by Izumi (1999), who found that fresh-cut carrots, bell peppers, spinach, Japanese radish, and potatoes treated by dipping in electrolyzed water (pH 6.8, 20 ppm available chlorine) for 3 min showed a reduction in the total microbial count of 0.6–2.6 log CFU/g. He also found that the electrolyzed water did not influence the tissue pH, surface color, or general appearance of the fresh-cut vegetables. González et al. (2004) reported that shredded carrots treated with 1000 ppm acidified sodium chlorite (ASC) showed at least a 3.27 log CFU/g reduction in the total aerobic count. However, the concentrations used by these authors were not able to maintain the quality of shredded carrots (shelf-life 4–6 days at 5 °C).

Three minutes of dipping was finally selected to measure the effects of different dipping temperatures (1, 20, 40 and 50 °C). Regardless of the dipping temperature, a reduction of 0.29–2.67 log CFU/g (Fig. 2A) and 0.22–2.60 log CFU/g (Fig. 2B) were achieved for total bacteria and yeast and fungi, respectively, in carrots treated with the aforementioned sanitizers. Table 2 also showed the effects of dipping temperatures on the efficacy of AlEW and CA alone or in combination in the reduction of the total bacteria, yeast and fungi on carrot. Washing with AlEW (1.17 log reduction of total counts) and 1% citric acid (1.7 log reduction of total counts) at 1 °C for 3 min showed significantly greater ($p \leq 0.05$) sanitizing effect than DIW treatment (0.29 log reduction of total counts) compared to control, while sanitizing effect with AlEW (1.78 log reduction of total counts) and 1% citric acid (2.37 log reduction of total counts) was much more enhanced than DIW treatment (0.76 log reduction of total counts) at 20 °C. Similar results were observed in yeast and fungi at each treatment. The reductions in microflora significantly increased ($p \leq 0.05$) as the dipping temperature increased from 1 to 50 °C for each treatment. It has been reported that mild heat treatment improved the efficacy of a sanitizer at killing or removing microorganisms on produce. These mild heat treatments consisted of subjecting the products to temperatures of 50–90 °C for periods of time not exceeding 1–5 min (Orsat et al., 2001). Klaiber et al. (2005) found that washing uncut carrots with cold chlorinated water (200 mg/L, 4 °C) and warm tap water (50 °C) reduced aerobic mesophilic bacteria by 1.7 and 2.0 log CFU/g, respectively, while

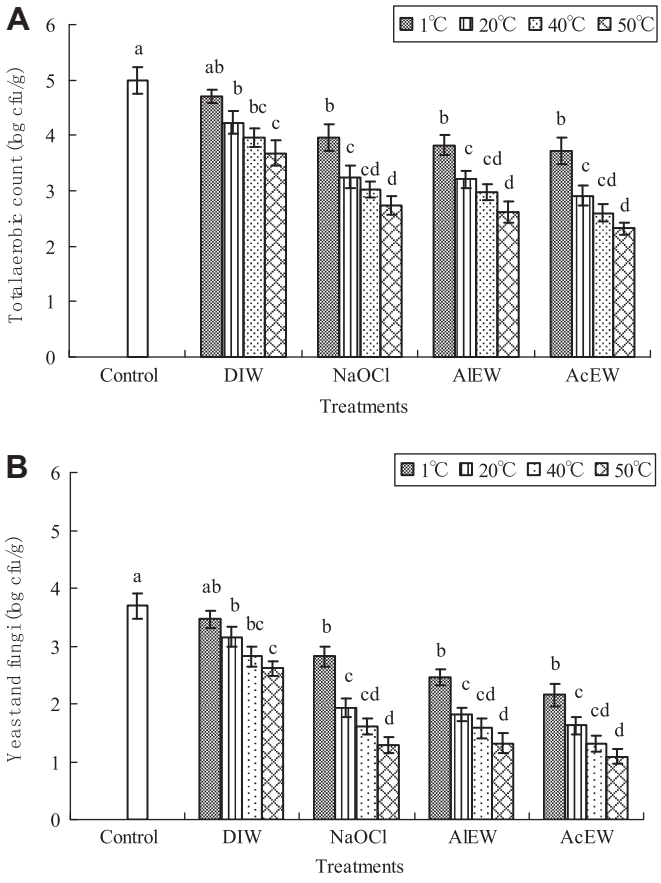

Fig. 2. Inactivation effect of electrolyzed water on the total counts (A), and yeast and fungi (B) in carrots treated at different dipping temperatures for 3 min. Vertical bars represent mean ± standard error of mean, $n = 6$. Bars labeled with different letters in the same treatment indicate a significant difference ($p \leq 0.05$). (Control: not treated; DIW: deionized water; NaOCl: 100 mg/L sodium hypochlorite; AlEW: alkaline electrolyzed water; AcEW: acidic electrolyzed water).

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Table 2
Effect of alkaline electrolyzed water and citric acid, either alone or in combination, on the inactivation of total counts, and yeast and fungi on carrots at different dipping temperatures for 3 min.

Treatment	Temperature (°C)	Total count (log CFU/g)	Yeast and fungi (log CFU/g)
Control	–	4.99 ± 0.16Aa[a]	3.69 ± 0.17Aa
DIW	1	4.70 ± 0.18ab	3.47 ± 0.17ab
	20	4.23 ± 0.21b	3.16 ± 0.21b
	40	3.97 ± 0.18bc	2.82 ± 0.18bc
	50	3.68 ± 0.16Bc	2.61 ± 0.19Bc
AlEW	1	3.82 ± 0.14b	2.46 ± 0.11b
	20	3.21 ± 0.10c	1.81 ± 0.10c
	40	2.97 ± 0.15cd	1.58 ± 0.12cd
	50	2.62 ± 0.12Cd	1.32 ± 0.14Cd
CA	1	3.29 ± 0.11b	2.02 ± 0.18b
	20	2.62 ± 0.16c	1.45 ± 0.10c
	40	2.31 ± 0.15cd	1.19 ± 0.21cd
	50	2.03 ± 0.21Cd	<1.0
AlEW + CA	1	2.49 ± 0.18b	1.23 ± 0.17b
	20	1.82 ± 0.19c	<1.0
	40	1.53 ± 0.11cd	<1.0
	50	1.28 ± 0.13Dd	NE

Control (not treated), DIW (deionized water), AlEW (alkaline electrolyzed water), CA (1% citric acid), and AlEW + CA (alkaline electrolyzed water + 1% citric acid).
<1.0 – Not detectable on direct plate count, but positive on enrichment media.
NE – Negative on enrichment.
[a] Values are mean ± standard error of mean, $n = 6$. Means within a column for each treatment bearing different lowercase letters are significantly different ($p \leq 0.05$). Means within the same column with different capital letters are significantly different ($p \leq 0.05$) between all treatments at 50 °C compared to control.

washing with warm chlorinated water (200 mg/L, 50 °C) resulted in a 2.3 log CFU/g reduction. Shredded lettuce treated with chlorinated water for 3 min at 47 °C showed a reduction in the aerobic bacterial count of 3 log CFU/g, while a reduction of approximately 1 log CFU/g was observed in response to the same treatment at 4 °C (Delaquis et al., 1999). Koseki et al. (2004) found that the appearance of mildly heated (50 °C) lettuce did not deteriorate after treatment. They also found that the application of AlEW as a prewash agent was useful, and that combined treatment with AlEW and AcEW was effective. Mild heat treatment of fresh produce has been reported to enhance the bactericidal effect of sanitizers and the physiological and sensory quality of the produce. A significantly more enhanced anti-browning effect was observed (Jin et al., 2009) when the lettuce leaves were subjected to both 1% citric acid and AlEW treatment at temperatures greater than 40 °C. Conversely, our results revealed that combined 1% citric acid and AlEW treatment at 50 °C resulted in a significant ($p \leq 0.05$) reduction of the total bacterial counts (3.71 log CFU/g) and yeast and fungi (3.69 log CFU/g) on shredded carrots when compared to each single treatment alone (Table 2).

Based on our experimental data derived from the combined effect of AlEW and 1% CA against background microflora (Table 2), the optimal condition (3 min at 50 °C) was selected to determine the combined effect on the reduction of *L. monocytogenes* and *E. coli* O157:H7 inoculated on shredded carrots (Fig. 3A and B). Dipping shredded carrots in DIW for 3 min at 50 °C for *L. monocytogenes* reduced the populations by 1.33 log CFU/g, while treatment with 100 ppm NaOCl, AlEW, and 1% CA alone decreased these populations by 2.30, 2.70, and 2.81 log CFU/g, respectively (Fig. 3A). Combined treatment with AlEW and 1% CA significantly ($p \leq 0.05$)

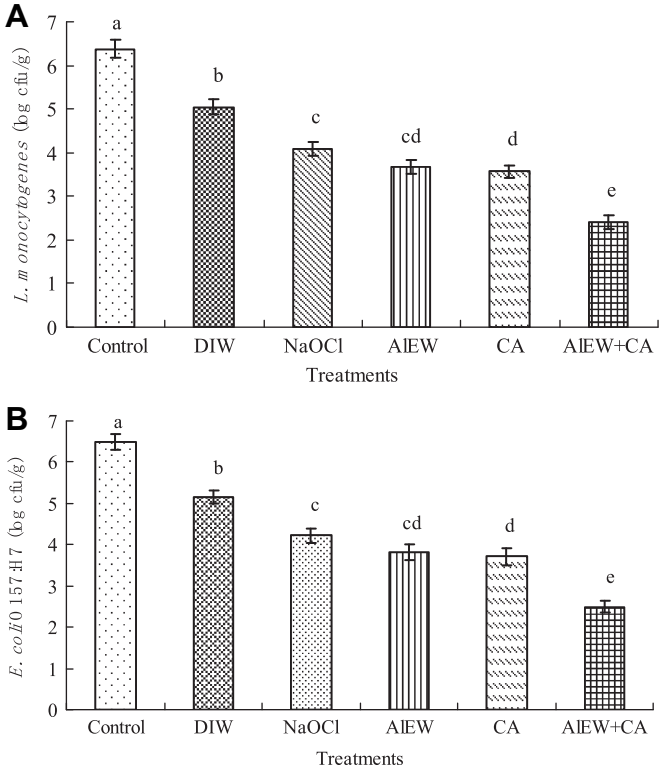

Fig. 3. Effect of alkaline electrolyzed water and citric acid either alone or in combination on the inactivation of *L. monocytogenes* (A) and *E. coli* O157:H7 (B) on carrots when applied at 50 °C for 3 min. Vertical bars represent mean ± standard error of mean, $n = 6$. Bars labeled with different letters among treatments indicate a significant difference ($p \leq 0.05$) (Control: not treated; DIW: deionized water; NaOCl: 100 mg/L sodium hypochlorite; AlEW: alkaline electrolyzed water; CA: 1% citric acid; AlEW + CA: alkaline electrolyzed water + 1% citric acid).

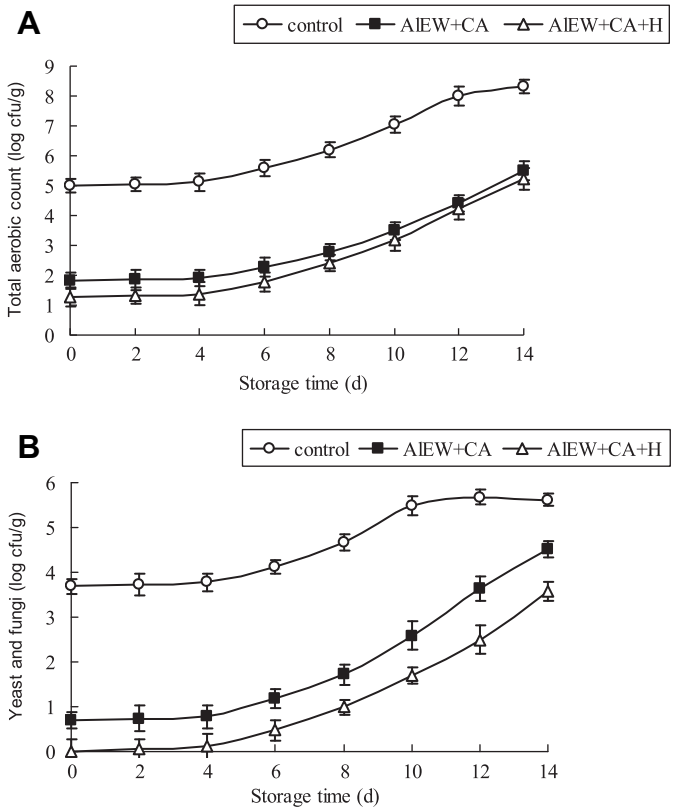

Fig. 4. Effect of combined treatment with alkaline electrolyzed water (AlEW) and Citric acid (CA) with or without mild heat (H) on the total aerobic counts (A) and yeast and fungi (B) on carrots during storage at 4 °C. Values shown are mean ± standard error of mean, $n = 6$. The error bars indicate 95% confidence intervals.

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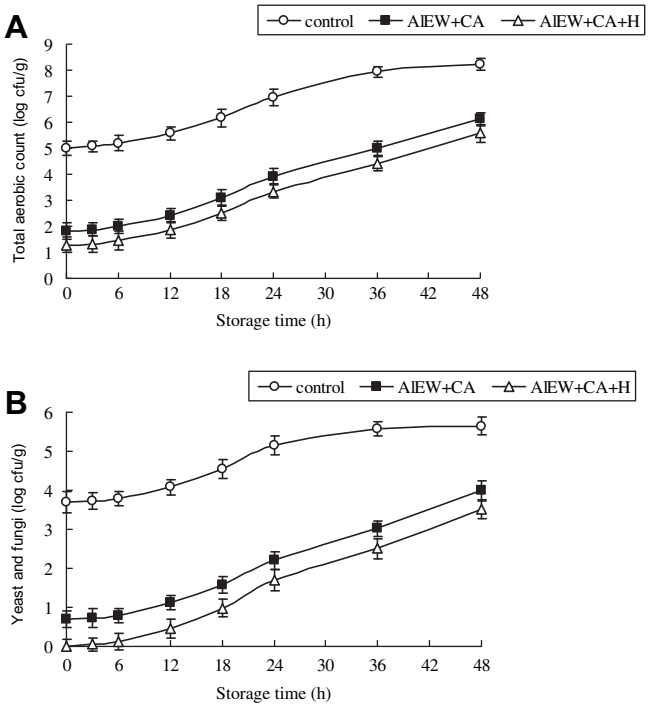

Fig. 5. Effect of combined treatment with alkaline electrolyzed water (AlEW) and citric acid (CA) with or without mild heat (H) on the total aerobic counts (A), and yeast and fungi (B) on carrots during storage at 25 °C. Values shown are mean ± standard error of mean, $n = 6$. The error bars indicate 95% confidence intervals.

decreased the populations of *L. monocytogenes* by 3.97 log CFU/g when compared with each treatment alone. Similar reductions in the levels of *E. coli* O157:H7 on carrots were observed under the same conditions (Fig. 3B). Specifically, treatment with DIW, NaOCl, AlEW, and 1% CA alone decreased the populations by 1.35, 2.27, 2.66, and 2.78 log CFU/g, respectively, but combined treatment with AlEW and 1% CA showed the strongest ($p \leq 0.05$) inactivation of 4.0 log CFU/g compared to other treatment alone. Many sanitizers have been found to effectively reduce pathogens, including *E. coli* O157:H7, *Salmonella* spp. and *L. monocytogenes*, on fresh-cut carrot shreds (Singh et al., 2002; Ruiz-Cruz et al., 2007; González et al., 2004). Neutral electrolyzed water (NEW) has also been used (Abadias et al., 2008) against native microbiota on grated carrot and no changes in carrot appearance were noted. Acidified sodium chlorite (ASC) was found to be the most effective treatment for reducing pathogens on fresh-cut carrots at all concentrations evaluated (Ruiz-Cruz et al., 2007). Specifically, they found that ASC reduced the three pathogen populations to undetectable levels (with a 10 CFU/g detection limit), achieving reductions of 4.81, 4.84 and 2.5 log CFU/g for *E. coli*, *Salmonella* and *L. monocytogenes*, respectively. In addition, Park and Beuchat (1999) showed that treatment with 40 or 80 ppm peroxyacetic acid (PA) reduced the population of *Salmonella* by 2.6 log CFU/g. Many combinations of physical and chemical treatments have been tested to determine if they showed enhanced antimicrobial action in recent years. Effective combined use of AlEW and AcEW with mild heat was reported by Koseki et al. (2004). Conversely, AcEW combined with ozone was applied using sequential washes (Wang et al., 2004). Bari et al. (2005) investigated the efficacy of nisin and pediocin treatments in combination with EDTA, citric acid, sodium lactate, potassium sorbate and phytic acid for reducing *L. monocytogenes* on fresh-cut produce. Yuk et al. (2007) showed that ozone treatment produced reductions of *E. coli* O157:H7 and *L. monocytogenes* that were less than 1.0 and 0.5 log, respectively, on enoki mushroom. The efficacy was improved using combined application of 3 ppm ozone and 1% citric acid, which resulted in reductions of 2.26 and 1.32 log, respectively. It would be expected that combinations of sanitizers and/or other intervention methods would have additive, synergistic or antagonistic interactions (Parish and Davidson, 1993; Rajkowski and Baldwin, 2003).

The changes in microbial populations during the shelf-life study are shown in Figs. 4 and 5. Combined treatment with AlEW and 1% CA with mild heat treatment was found to effectively control the growth of aerobic bacteria, yeast and fungi during storage at 4 and 25 °C for 15 days and 48 h, respectively, when compared to unwashed controls. The end of the shelf-life was considered to occur when the total aerobic count was 7–8 log CFU/g and the total yeast count was 5 log CFU/g (Debevere, 1996; Gómez-López et al., 2007a; Alegria et al., 2010). The results of our study revealed that no microbial counts reached unacceptable levels after 15 days at 4 °C and 48 h of storage at 25 °C for treated carrots. The shelf-life of controls (untreated carrots) stored at 4 °C was limited to 10 days and 24 h stored when stored at 25 °C due to the occurrence of unacceptable counts of bacteria ($\geq 10^7$ CFU/g) and yeast and fungi ($\geq 10^5$ CFU/g) (Figs. 4A and B, 5A and B). This could have resulted in shelf-life extensions of five days and 24 h for treated carrots stored at 4 and 25 °C, respectively. Similarly, Gómez-López et al. (2007a) found a shelf-life extension of 4 days in response to the application of gaseous ClO_2 to minimally processed carrots. Conversely, a shelf-life extension of at least five and three days in samples stored at 4 and 7 °C, respectively, can be achieved by treating

Table 3
Sensory evaluation of shredded carrots treated with AlEW + CA with mild heat and stored at 4 °C for 15 days.

Quality attributes		Storage (days)					
		0	3	6	9	12	15
Color	Untreated	2.7 ± 0.3	2.5 ± 0.2	2.2 ± 0.1	1.9 ± 0.1	1.5 ± 0.2	1.2 ± 0.3
	Treated	3.0 ± 0.2	2.8 ± 0.2	2.6 ± 0.3	2.4 ± 0.1	2.2 ± 0.2	2.0 ± 0.1
Firmness	Untreated	1.3 ± 0.3	1.7 ± 0.2	2.3 ± 0.3	3.0 ± 0.4	**3.5** ± 0.3	**4.0** ± 0.6
	Treated	1.0 ± 0.2	1.3 ± 0.1	1.7 ± 0.4	2.1 ± 0.3	2.5 ± 0.2	3.0 ± 0.5
Fresh-like aroma	Untreated	1.3 ± 0.4	1.8 ± 0.3	2.2 ± 0.4	2.9 ± 0.5	**3.2** ± 0.3	**3.5** ± 0.4
	Treated	1.2 ± 0.3	1.5 ± 0.2	1.8 ± 0.3	2.2 ± 0.4	2.6 ± 0.5	2.9 ± 0.3
General acceptance	Untreated	1.2 ± 0.2	1.8 ± 0.3	2.4 ± 0.4	3.0 ± 0.5	**3.4** ± 0.4	**3.9** ± 0.6
	Treated	1.0 ± 0.1	1.3 ± 0.2	1.6 ± 0.3	2.0 ± 0.4	2.5 ± 0.5	3.0 ± 0.4

Values are the average of five observations (Mean ± SD). Numbers in bold are scores above the acceptability limit. Colour scoring system: 1 = very light orange; 2 = light orange; 3 = orange; 4 = dark orange; 5 = very dark orange, where anchor 3 corresponds to the initial carrot fresh appearance immediately after cutting.
Firmness scoring system: 1 = very firm, 2 = firm; 3 = moderate; 4 = soft; and 5 = very soft, where anchor 1 corresponds to the perception of the fresh-cut carrot immediately after cutting.
Fresh-like aroma scoring system: 1 = very intense, 2 = intense; 3 = moderate; 4 = low; and 5 = absent, where anchor 1 corresponds to the perception of the carrots aroma immediately after cutting.
General acceptance scoring system: 1 = excellent freshly cut, 2 = good, 3 = limit of marketability, 4 = fair limit of usability and 5 = poor unusable.

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shredded cabbage with neutral electrolyzed oxidizing water (Gómez-López et al., 2007b).

The sensorial quality of the shredded carrots was evaluated by a sensory panel throughout the storage period. The mean scores of sensory attributes (color, firmness, fresh-like aroma, general acceptance) for untreated and treated carrots are summarized in Table 3. Sensory attributes were distinguished by a panel using a 5-point numeric rating scale (Alegria et al., 2009). The general acceptance was fixed at a score of 3 (limit of marketability), and scores greater than 3 indicated unacceptable samples. The end of the shelf-life from the sensory point of view was attained when at least one of the mean scores was above the middle point of the respective scale. In the case of untreated samples and the firmness, fresh-like aroma and general acceptance reached a score of 3 after storage for nine days at 4 °C. These findings indicated that the shelf-life of untreated carrots was limited to nine days from the sensorial point of view. Moreover, the sensory attributes were acceptable for treated carrots, with the shelf-life being extended up to 15 days at refrigerated temperature. These results are in agreement with those of Chaudry et al. (2004), who noted that minimally processed carrots may be treated with a 2 kGy dose of gamma radiation to keep the appearance and flavor quality acceptable and extend the shelf-life up to 14 days at refrigerated temperature. Farkas et al. (1997) reported that a 1 kGy dose of gamma radiation was sufficient to reduce bacterial load and improve microbiological shelf-life, and that it extended the sensorial quality of precut peppers and carrots.

Based on the results of these studies, combined treatment with AlEW and 1% CA with mild heat appears to be a promising method to ensure microbial safety as well as to prolong the shelf-life of treated shredded carrots without impairing sensorial quality. The application of combined AlEW and CA for controlling microorganisms on carrots has not previously been reported. Therefore, the synergistic effect of AlEW and CA may provide valuable insight into the reduction of foodborne pathogens on fresh-cut produce. In general, combinations of chemical disinfectants maintain better sensory and microbial quality of the product. Therefore, more studies should be conducted to determine the synergistic effects of combining disinfection technologies.

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