Inactivation of *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* on plastic and wood kitchen cutting boards by neutral electrolyzed water

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Introduction

The use of cutting boards for food preparation in both the home and commercial food service operations is a common and established practice. However, contaminated wooden and plastic boards are a potential source of transmission of pathogens. Frequent cleaning and disinfection of food contact surfaces is an effective means to reduce cross contamination and the occurrence of food-borne disease outbreaks.

In recent years, electrolyzed water solutions have been introduced for application as sanitizers. These solutions are generated by electrolysis of a dilute NaCl solution passing through the anode of a membrane electrolyser (Figure 1), and contain active oxidizers like hypochlorous acid (Len *et al.*, 2000).

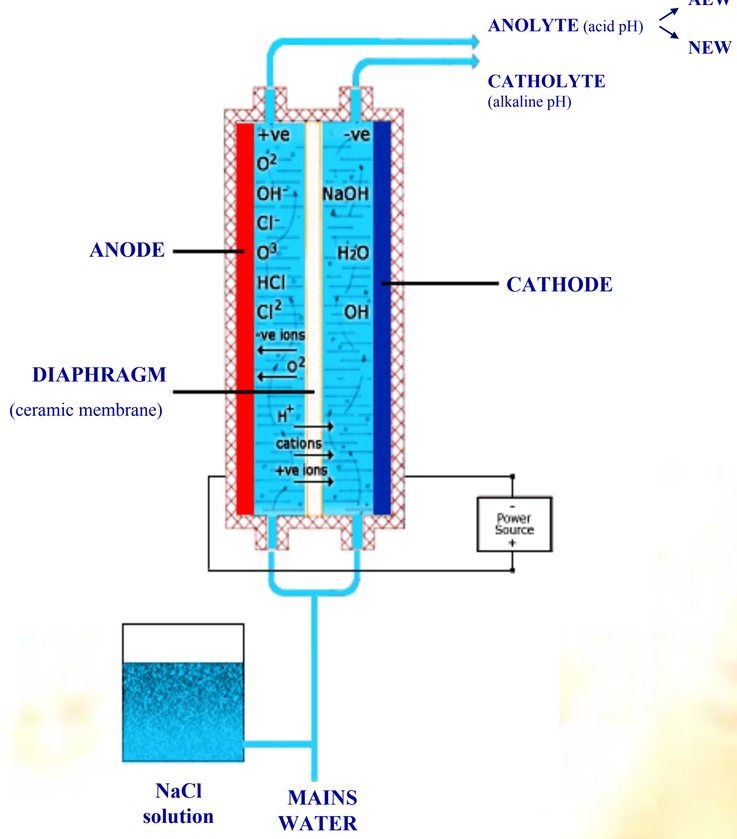

Figure 1. Diaphragmatic cell for electrolyzed water synthesis.

Methods

Bacterial cultures: *E. coli* CECT 405, *P. aeruginosa* CECT 116, *S. aureus* CECT 239 and *L. monocytogenes* CECT 4032 (Spanish Type Culture Collection) were cultured on TSA at 37ºC for 24 h. Bacteria were resuspended in tryptone- NaCl solution to obtain a suspension of 9 to 10 $\log_{10}$ CFU/ml .

Preparation of treatment solutions: NEW was generated using an Envirolyte el–900 unit (Envirolyte Industries LTD, Estonia) (Figure 2). A 25% sodium chloride solution and tap water were simultaneously pumped into the generator to obtain an amperage of 32 ± 2 A. Final NEW or NaClO solutions were prepared by diluting the concentrated solutions in deionized water.

Preparation and inoculation of surfaces: Plastic (polypropylene) and pine wood kitchen cutting boards bought at a local market, were cut in 50 cm^2 portions and sterilized in autoclave (plastic surfaces) or under UV light (wooden surfaces). Boards were immersed 5 min in the bacterial suspension and dried under sterile air. The initial population on the surface was obtained by swabbing one face of an inoculated surface with a sterile cotton swab and by inoculating onto duplicate TSA plates.

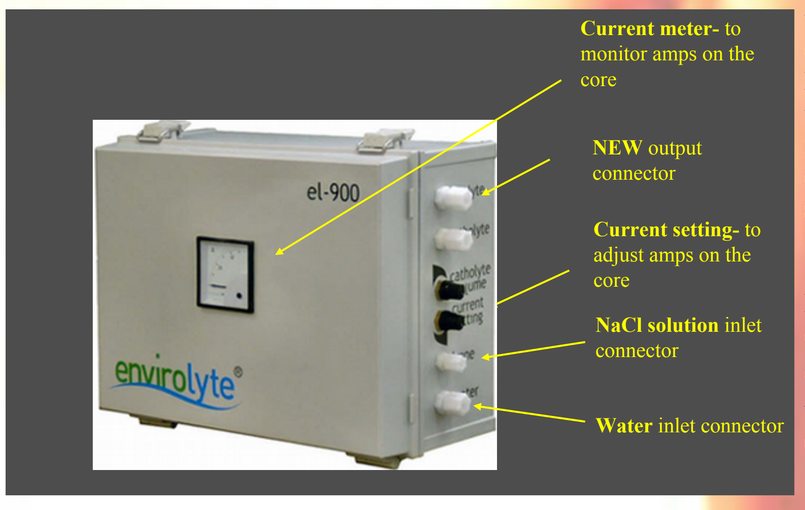

Figure 2. Neutral electrolyzed water generator

Aims

Previous studies in our laboratory have demonstrated that rinsing in neutral electrolyzed water (NEW) is an effective method to control the presence of pathogenic bacteria on the surface of fresh tomatoes (Deza *et al.* 2003) and significantly reduces the presence of pathogenic and spoilage bacteria on stainless steel and glass surfaces (Deza *et al.* in press).

The aim of this work was to evaluate the efficacy of NEW in reducing populations of *E. coli, P. aeruginosa, S. aureus* and *L. monocytogenes* on plastic and wooden cutting boards. Its effectiveness was compared with that of a sodium hypochlorite solution (NaClO).

Washing treatment: Inoculated surfaces were rinsed for 1 min (plastic boards) or 1 and 5 min (wooden boards) in 250 ml of either NEW, NaClO solution or deionized water (control). After the treatment, the population of each strain was determined on the surface by swabbing. The swab was washed in 5 ml of neutralizing solution (sodium thiosulfate 0.5 %) and appropriate dilutions of this solution were plated onto TSA plates.
Immediately after removing the surface from the solution, 20 % sodium thiosulfate was added to the washing solutions. After 5 min of neutralization, the number of viable cells in the washing solutions were determined on TSA plates. For enrichment, 5 ml of each treatment solution was transferred to 50 ml of TSB and incubated at 37 ºC for 24 h.

Data analysis: All trials were repeated at least four times. Microbial counts were expressed as $\log_{10}$ CFU /ml (washing solutions and inocula) or $\log_{10}$ CFU/50cm^2 (surface). Data were subjected to analysis of variance and Duncan's multiple range test using STATGRAPHICS (Statistical Graphics Corporation, Englewood Cliffs, U.S.A.).

Results and Discussion

Properties (pH, ORP and active chlorine concentration) of treatment solutions used in the study are shown in Table 1.

Table 1 Physicochemical properties of tested solutions*

Treatment solution	pH	ORP (mV)	Active chlorine (mg/l)
Deionized water (control)	6.42 ± 0.53	648.0 ± 09.0	0
NEW	7.76 ± 0.35	775.0 ± 10.0	64.1 ± 4.5
NaClO solution	8.11 ± 0.41	738.0 ± 15.0	62.3 ± 5.6

* values are the means of at least 16 measurements ± SD. NEW: neutral electrolyzed water. ORP: oxidation-reduction potential.

Washing of contaminated cutting boards in NEW for 1 min, revealed a significant difference ($P \leq 0.05$) between the decontamination of plastic and wooden surfaces: After treating plastic boards, initial populations of approx. 7.5 $\log_{10}$ CFU/50 cm^2 decreased by about 5.4 logs (Fig. 3). In wooden boards instead, the same treatment reduced the initial bacterial populations by 2.5 $\log_{10}$ CFU/50 cm^2 (Fig. 4 A).

Taking these results into account, the exposure time for rinsing wood surfaces was increased to 5 min, obtaining reductions of about 4 $\log_{10}$ CFU/50 cm^2 after soaking in NEW (Fig. 4 B).

The difference between type of surface was also observed in the control treatment: washing contaminated plastic surfaces with sterile deionized water decreased the populations of all strains by about 1.7 $\log_{10}$ CFU/50cm^2 (Fig. 3) whereas on wooden boards the same treatment yielded no significant differences in reduction of the population of all strains (Fig. 4).

These findings are in agreement with the results of Boucher et *al.* 1998 and Welker *et al.* 1997, in where wooden cutting boards can absorb moisture —and contaminating bacteria— into the porous structure of the material, making it more difficult to clean.

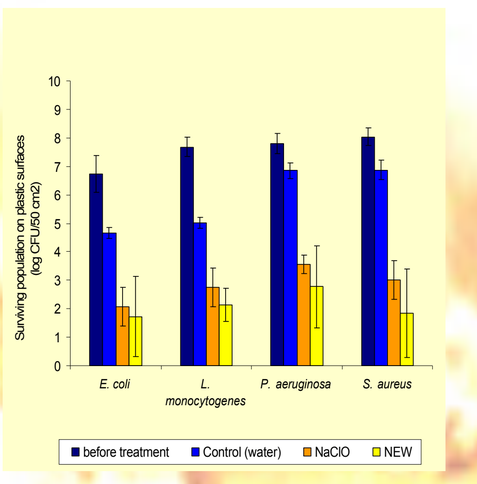

Figure 3. Inactivation of *E. coli*, *L. monocytogenes, P. aeruginosa*, and *S. aureus* on **plastic** cutting boards after 1 min treatment with neutral electrolyzed water (NEW) and sodium hypochlorite solution (NaClO) at 23 ± 2 ºC. Results are means ± SD (n = 4)

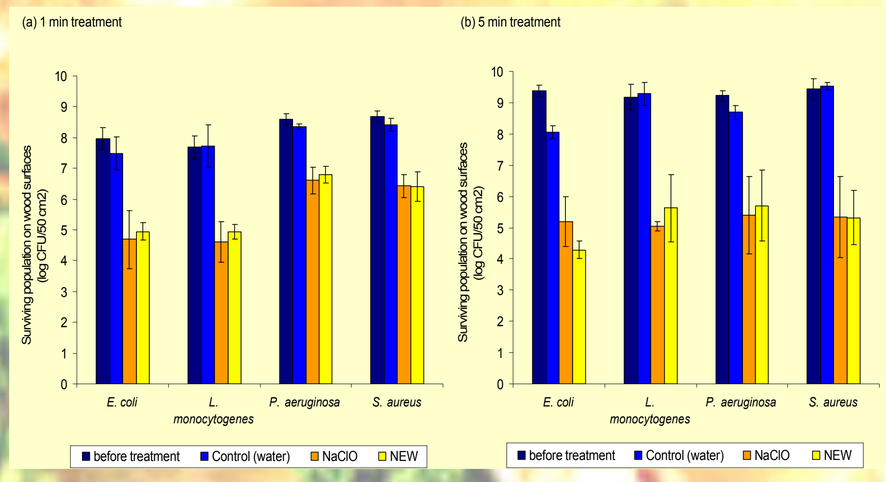

Figure 4. Inactivation of *E. coli*, *L. monocytogenes, P. aeruginosa*, and *S. aureus* on **wood** cutting boards after washing for 1 min **(a)** or 5 min **(b)** with neutral electrolyzed water (NEW) and sodium hypochlorite solution (NaClO) at 23 ± 2 ºC. Results are means ± SD (n = 4)

Sodium hypochlorite is one of the most widely used disinfectants in household sites and food industries. In this study, NEW efficacy in reducing bacterial populations on plastic and wooden surfaces was comparable to that of a NaClO solution of similar pH, ORP and active chlorine concentration.

Disinfection with NEW has the advantage of being generated on-site, reducing in this way transportation, storage and mixing of chemicals; and it is potentially less hazardous for workers. Hence, it represents an advantageous alternative to NaClO for the disinfection of food contact surfaces.

The mean surviving populations of all bacteria in NEW and NaClO washing solutions were <1 $\log_{10}$ CFU/ml after soaking both surfaces for 1 min, whereas in control water, an average of 6.45 $\log_{10}$ CFU/ml of all strains was recovered. In wooden boards, after a 5 min treatment with NEW or NaClO, no survivors were detected in washing solutions by a direct plating procedure.

Conclusions

In summary, NEW reveals to be as effective as NaClO to significantly reduce the presence of the evaluated pathogenic bacteria on plastic and wooden kitchen cutting boards, with the advantage of being a safe and easy-to-handle option.

By the other hand, the obtained results show that wooden boards are not a suitable option for food preparation since they are very difficult to clean.

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Inactivation of *Escherichia coli* O157:H7, *Salmonella enteritidis* and *Listeria monocytogenes* on the surface of tomatoes by neutral electrolyzed water

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ABSTRACT

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Aims: To determine the efficacy of neutral electrolyzed water (NEW) in killing *Escherichia coli* O157:H7, *Salmonella enteritidis* and *Listeria monocytogenes*, as well as nonpathogenic *E. coli*, on the surface of tomatoes, and to evaluate the effect of rinsing with NEW on the organoleptic characteristics of the tomatoes.

Methods and Results: The bactericidal activity of NEW, containing 444 or 89 mg l^{-1} of active chlorine, was evaluated over pure cultures (8·5 log CFU ml^{-1}) of the above-mentioned strains. All of them were reduced by more than 6 log CFU ml^{-1} within 5 min of exposure to NEW. Fresh tomatoes were surface-inoculated with the same strains, and rinsed in NEW (89 mg l^{-1} of active chlorine) or in deionized sterile water (control), for 30 or 60 s. In the NEW treatments, independent of the strain and of the treatment time, an initial surface population of about 5 log CFU $sq.cm^{-1}$ was reduced to <1 log CFU $sq.cm^{-1}$, and no cells were detected in the washing solution by plating procedure. A sensory evaluation was conducted to ascertain possible alterations in organoleptic qualities, yielding no significant differences with regard to untreated tomatoes.

Significance and Impact of the Study: Rinsing in NEW reveals as an effective method to control the presence of *E. coli* O157:H7, *S. enteritidis* and *L. monocytogenes* on the surface of fresh tomatoes, without affecting their organoleptic characteristics. This indicates its potential application for the decontamination of fresh produce surfaces.

Keywords: disinfectant, *E. coli* O157:H7, *L. monocytogenes*, neutral electrolyzed water, organoleptic quality, rinsing fresh tomatoes, *S. enteritidis*.

INTRODUCTION

Fruits and vegetables can become contaminated with pathogenic micro-organisms while growing in fields, during harvesting and postharvest handling, processing and distribution (Beuchat 1996). Human gastroenteritis has been epidemiologically linked to the consumption of ready-to-eat salads contaminated with enterotoxigenic *Escherichia coli* (Abdul-Raouf *et al.* 1993) and *Listeria monocytogenes* (Beuchat and Brackett 1991); outbreaks of salmonellosis have been attributed to the consumption of contaminated tomatoes (Zhuang *et al.* 1995; Beuchat 1996). Also, the growth of *L. monocytogenes* and *Salmonella* spp. on the surface of whole fresh-cut tomatoes has been reported (Asplund and Nurmi 1991; Beuchat and Brackett 1991).

Washing fresh produce with running tap water may remove soil and other debris, but it has a limited effect on surface micro-organisms that occur at populations ranging from 10^3 to 10^9 CFU g^{-1} (Koseki *et al.* 2001). A variety of disinfectants (chlorine, hydrogen peroxide, organic acids, ozone, etc.) have been used to reduce the bacterial population on fruits and vegetables. However, besides their potential toxicity, they cannot completely remove or

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inactivate micro-organisms on fresh produce (Koseki and Itoh 2001; Park *et al.* 2001).

In recent years, acidic electrolyzed water (AEW) and neutral electrolyzed water (NEW) have been introduced for application as sanitizers. These solutions are generated by electrolysis of a dilute NaCl solution passing through the anode of a membrane electrolyser. AEW has a strong bactericidal effect on most known pathogenic bacteria due to its low pH (2–4) and high oxidation–reduction potential (ORP > 1000 mV), and because it contains active oxidizers like hypochlorous acid (Kim *et al.* 2000b; Len *et al.* 2000), it is effective in killing food-borne pathogens *in vitro* conditions (Venkitanarayanan *et al.* 1999b; Kim *et al.* 2000a) and in reducing microbial counts and pathogens in vegetables (Koseki *et al.* 2001; Koseki and Itoh 2001; Park *et al.* 2001; Bari *et al.* 2003; Kim *et al.* 2003).

NEW is generated like AEW, but a part of the product formed at the anode is redirected into the cathode chamber, thus increasing the content of ClO^- ions. Because of its neutral pH, NEW does not contribute as aggressively as AEW to the corrosion of processing equipment or irritation of hands, and is more stable as chlorine loss is significantly reduced at pH 6–9 (Rojas and Guevara 2000; Len *et al.* 2002). Izumi (1999) has evaluated the effect of NEW (pH 6·8 and 20 mg l^{-1} active chlorine) on total microbial count in fresh-cut vegetables, obtaining reductions up to 2·6 log CFU g^{-1} without significant effect on tissue pH, surface colour and general appearance of vegetables.

The aim of this work was to determine the effectiveness of NEW in killing *E. coli* O157:H7, *Salmonella enteritidis*, *L. monocytogenes* and nonpathogenic *E. coli*, *in vitro* and on the surface of tomatoes, with a view to its potential application to fresh produce and food contact surfaces as an antimicrobial treatment. A sensory evaluation was also performed in order to evaluate the effect of rinsing with NEW on the organoleptic characteristics of the tomatoes.

MATERIALS AND METHODS

Preparation of treatment solutions

NEW was generated using a Eurostel EE-90 unit (Aquastel Balti OU, Tallinn, Estonia). A 25% sodium chloride solution and tap water were simultaneously pumped into the generator to obtain amperage of 32 ± 2 A. For this study, NEW (containing approx. 444 mg l^{-1} of active chlorine) was diluted 1 : 5 in deionized sterile water, to obtain a final active chlorine concentration of about 89 mg l^{-1}. Deionized sterile water was also used as control.

pH, ORP and active chlorine concentration were determined for both treatment solutions. The former magnitudes were measured after preparation, using a pH/ion/conductivity meter (CRISON micro-pH 2001) with a pH electrode (CRISON, 52–11) and an ORP electrode (CRISON platinum Ag/AgCl electrode, 52–61). The latter, by an iodometric method (APHA 1998).

Treatment of pure culture

The strains used for this study were obtained from the Spanish Type Culture Collection (CECT): *E. coli* CECT 405 (ATCC strain 10536, proposed for testing antibiotics, antimicrobial preservatives and chemotherapeutic agents), *E. coli* O157:H7 CECT 4267 (ATCC strain 35150, isolated from an outbreak of haemorrhagic colitis, produces Shiga-like toxin I and II) *S. enteritidis* CECT 556 (isolated from water in Valencia, Spain) and *L. monocytogenes* CECT 4032 (isolated from soft cheese, associated with a case of meningitis). Strains were cultured on TSA plates [Tryptone Soy Broth (Panreac Química S.A., Barcelona, Spain) with the addition of 15 g l^{-1} agar no. 3 (Oxoid, Basingstoke, Hampshire, UK)] at 37°C for 24 h.

The efficiency of NEW to produce a reduction in at least 5 logs in viable cell counts (bactericidal activity) in clean conditions was evaluated according to the European Standard UNE-EN 1276 (Anonymous 1998). One millilitre of bacterial culture of about 8·5 log CFU ml^{-1} was transferred to sterile tubes together with 1 ml of sterile water. Eight millilitre of pure NEW (444 ± 8·15 mg l^{-1} active chlorine) or diluted 1 : 5 in deionized water (89 ± 7·5 mg l^{-1} active chlorine) were added. The tubes were hand-shaken to mix the resultant suspension, and incubated at room temperature (23 ± 2°C) for 5 min. Deionized water was used as a control.

Following treatment, 1 ml of each sample was transferred to 9 ml of neutralizing solution (sodium thiosulphate 0·5%) and the suspension hand shaken. After 5 min of neutralization, 1 ml of the appropriate dilution 1 : 10 in tryptone sodium chloride solution (pH 7·2 ± 0·2) was pour plated on TSA. The plates were incubated at 37 ± 1°C for 24 h. The experiment was repeated four times.

Preparation and inoculation of tomatoes

Tomatoes (*Lycopersicum esculentum* var. Durinta) were purchased at a local supermarket and stored at 4°C , for a maximum of 3 days before testing. Units of similar size (70–80 g) without lesions on skin were used. Their surface area was calculated in order to obtain the number of CFU $sq.cm^{-1}$. Tomatoes were first washed with tap water for 1 min and air-dried under sterile air in a laminar flow cabinet for 15 min in individual metallic strainers.

For the inoculation of tomatoes, a bacterial suspension of 8·98–9·23 log CFU ml^{-1} was prepared using 70 ml of tryptone sodium chloride solution. The bacterial population

of each inoculum was confirmed either by pouring 1 ml (for *E. coli*) or by surface-plating 0·1 ml (for *E. coli* O157:H7, *S. enteritidis* and *L. monocytogenes*) of appropriate dilutions of the suspension (using the same solution) on duplicate selective plates, using Coli ID medium (bioMérieux, Marcy l'Etoile, France) for *E. coli*, Sorbitol-MacConkey agar (Merck, Darmstadt, Denmark) for *E. coli* O157:H7, XLD agar (Oxoid) for *S. enteritidis*, and PALCAM agar (Merck) for *L. monocytogenes*. Plates of Coli ID, Sorbitol-MacConkey and XLD agar were incubated at 37°C for 24 h, and plates of PALCAM agar at 37°C for 48 h.

Tomatoes were immersed for 1 min in the bacterial suspension of 9 log CFU ml^{-1}, and then dried in individual sterile metallic strainers under sterile air in a laminar flow cabinet for 15 min at room temperature (23 ± 2°C).

Treatment and bacteriological analysis of tomatoes

The initial population on tomato surface was obtained by swabbing the whole surface of an inoculated air-dried tomato with a sterile cotton swab moistened with 5 ml of sterile tryptone sodium chloride solution. Appropriate dilutions of this solution were plated onto selective plates as described above. Inoculated tomatoes were placed in individual sterile bags containing 100 ml of electrolyzed neutral water diluted 1 : 5, or sterile deionized water (control). The bags were shaken vigorously by hand for 30 or 60 s. After immersion in the treatment or control water, tomatoes were removed with a sterile metallic strainer and allowed to drain completely. The whole surface of each tomato was then swabbed with a sterile cotton swab. The swab was washed in 5 ml of neutralizing solution and appropriate dilutions of this solution were plated onto selective plates. A volume of 1 ml of the treatment or control water was also transferred to 9 ml of neutralizing solution and appropriate dilutions were plated onto selective plates, as described in 'Preparation and inoculation of tomatoes'. All the experiments were conducted at room temperature (23 ± 2°C), in order to imitate normal washing procedures for unprocessed produce at home.

Sensory evaluation

The organoleptic properties of un-inoculated tomatoes treated with NEW (pure or diluted 1 : 5 in water) and untreated (washed with tap water) was evaluated by 12 panellists. Tomatoes were washed under tap water for 1 min, drained and submitted for 1 min to the above-described treatment solutions, and air-dried for 6 h at 23 ± 2°C. Panellists individually evaluated appearance, colour and taste of treated and untreated tomatoes. The quality evaluation was based on a five-point scale: 1, not acceptable; 2, limited quality; 3, normal; 4, good; 5, very good.

Data analysis

All trials were repeated four times. Microbial counts were expressed as log CFU ml^{-1} (washing solutions and inocula) or CFU $sq.cm^{-1}$ (tomato surface). The reported values of plate count or physicochemical properties are the mean values over four individual trials ± standard deviations. Sensory evaluation values represent the mean of 12 values ± standard deviations. Data were subjected to analysis of variance and Duncan's multiple range test using STATGRAPHICS (Statistical Graphics Corporation, Englewood Cliffs, NJ, USA). Significant differences in plate count data and in sensory evaluation were established by the least significant difference at the 0·05 level of significance.

RESULTS

The pH, ORP and active chlorine concentration of treatment solutions used for each strain, are shown in Table 1.

All the strains treated for 5 min with NEW (containing 444 or 89 mg l^{-1} active chlorine) were reduced by more than 6 log CFU mg l^{-1}, as determined by plating procedure using the European Standard UNE-EN 1276 (Table 2). No reduction in bacterial counts was achieved in the control samples.

Table 3 shows the inactivation of *E. coli*, *E. coli* O157:H7, *S. enteritidis* and *L. monocytogenes* on tomato surface treated with NEW. The initial population on tomato surface after inoculating and drying under cabinet for 15 min was between 5·29 and 5·58 log CFU $sq.cm^{-1}$. Washing with deionized water (control) reduced viable cells in all strains by approx. 2 log CFU $sq.cm^{-1}$ within 30 or 60 s.

Under treatment with NEW, the populations on tomato surface of all strains were reduced by an average of 4·18 log CFU $sq.cm^{-1}$ in 30 s, and 4·74 log CFU $sq.cm^{-1}$ in 60 s. Populations of *E. coli* O157:H7, *S. enteritidis* and *L. monocytogenes* on the surface of tomatoes showed no significant difference between treatments with NEW at 30 or 60 s, whereas reduction in nonpathogenic *E. coli* population after treatment for 60 s was significantly lower ($P \leq 0{\cdot}05$) than after treatment for 30 s. Also, the populations of all strains, either after 30 s or after 60 s, were very similar, without significant strain dependence.

The surviving population in the washing solutions (NEW diluted 1 : 5 or deionized water) is also indicated in Table 3. Under treatment with NEW, no survivors were detected by plating procedure. In control water, an average of 5·35 log CFU ml^{-1} was recovered.

Table 1 Physicochemical properties of tested solutions*

Strain used in each treatment	Deionized water			NEW			NEW (diluted 1 : 5)		
	pH	ORP (mV)	Cl (mg l^{-1})	pH	ORP (mV)	Cl (mg l^{-1})	pH	ORP (mV)	Cl (mg l^{-1})
E. coli	6·01 ± 0·10	587 ± 9·0	0	8·13 ± 0·11	803 ± 11	430·6 ± 9·0	7·99 ± 0·21	750 ± 10	86·12 ± 7·2
E. coli O157:H7	5·92 ± 0·56	551 ± 4·0	0	8·03 ± 0·23	816 ± 9·0	432 ± 5·1	8·15 ± 0·20	771 ± 7·0	86·40 ± 4·1
S. enteritidis	5·82 ± 0·23	575 ± 15	0	7·99 ± 0·15	795 ± 10	465 ± 7·5	8·19 ± 0·30	745 ± 8·0	93·00 ± 9·0
L. monocytogenes	6·30 ± 0·15	662 ± 9·0	0	8·2 ± 0·09	808 ± 7·5	450 ± 11	8·09 ± 0·05	760 ± 11	92·10 ± 10

*Values are mean ± S.D. of four repeated measurements.
NEW, neutral electrolyzed water; ORP, oxidation–reduction potential; Cl, active chlorine.

Table 2 Inactivation of *E. coli*, *E. coli* O157:H7, *S. enteritidis* and *L. monocytogenes* in pure culture by NEW (444 and 89 mg l^{-1} active chlorine) in 5 min at 23 ± 2°C

Strain	Initial population (log CFU ml^{-1})	Surviving population after 5-min treatment (log CFU ml^{-1})		
		Control (deionized water)	NEW (444 ± 8·15 mg l^{-1} active chlorine)	NEW (dilution 1 : 5) (89 ± 7·5 mg l^{-1} active chlorine)
E. coli	7·51 ± 0·11	7·50 ± 0·12	<1	<1
E. coli O157:H7	7·45 ± 0·04	7·46 ± 0·13	<1	<1
S. enteritidis	7·70 ± 0·18	7·62 ± 0·17	<1	<1
L. monocytogenes	7·51 ± 0·17	7·53 ± 0·21	<1	<1

Table 3 Inactivation of *E. coli*, *E. coli* O157:H7, *S. enteritidis* and *L. monocytogenes* on tomato surface by NEW (89 mg l^{-1} active chlorine) at 23 ± 2°C*

Strain	Treatment (s)	Inoculum (log CFU ml^{-1})	Surviving population on tomato surface (log CFU $sq.cm^{-1}$)			Surviving population in washing solution (log CFU ml^{-1})		Reduction in bacterial count (log CFU $sq.cm^{-1}$)	
			Initial population (no treatment)	NEW treatment (dilution 1 : 5)	H_2O treatment (control)	NEW (dilution 1 : 5)	H_2O (control)	NEW (dilution 1 : 5)	H_2O (control)
E. coli	30	8·98 ± 0·30	4·93 ± 0·69	0·87 ± 0·66	3·05 ± 0·13	<1	4·60 ± 1·13	4·06 ± 0·07	1·88 ± 0·77
	60	9·23 ± 0·05	5·53 ± 0·25	0·52 ± 0·58	3·36 ± 0·33	<1	5·54 ± 0·60	5·01 ± 0·46	2·17 ± 0·33
E. coli O157:H7	30	9·06 ± 0·15	5·46 ± 0·22	1·11 ± 0·87	3·24 ± 0·64	<2	5·39 ± 0·37	4·35 ± 0·72	2·22 ± 0·45
	60	9·06 ± 0·15	5·46 ± 0·22	0·54 ± 0·50	3·44 ± 0·49	<2	5·77 ± 0·55	4·92 ± 0·44	2·02 ± 0,51
S. enteritidis	30	9·02 ± 0·11	5·16 ± 0·24	1·49 ± 0·24	3·31 ± 0·07	<2	5·05 ± 0·38	3·67 ± 0·26	1·85 ± 0·31
	60	9·02 ± 0·11	5·16 ± 0·24	0·86 ± 0·67	2·96 ± 0·18	<2	5·29 ± 0·42	4·30 ± 0·75	2·20 ± 0·43
L. monocytogenes	30	9·01 ± 0·18	5·34 ± 0·35	0·73 ± 0·61	3·15 ± 0·64	<2	5·49 ± 0·34	4·66 ± 0·74	2·19 ± 0·85
	60	9·01 ± 0·18	5·34 ± 0·35	0·54 ± 0·37	2·69 ± 0·54	<2	5·60 ± 0·34	4·74 ± 0·69	2·65 ± 0·84

*Values are the mean of at least four repeated measurements ± S.D.

No significant differences ($P \leq 0·05$) were found in the sensory evaluation of uninoculated tomatoes washed with NEW (pure or diluted 1 : 5) or with tap water. On an ascending five-point scale, for both treated and control tomatoes, the mean values were between 3·21 and 3·54 for appearance, between 3·25 and 3·96 for smell, and between 3·08 and 3·83 for taste.

DISCUSSION

In this study, the bactericidal effectiveness of 1 : 5 diluted NEW, both in pure culture and on the surface of tomatoes, has been assessed on four bacterial strains. Three of them (*E. coli* O157:H7, *S. enteritidis* and *L. monocytogenes*) are food-borne pathogens reported to be present in vegetables

(including tomatoes), whose detection in food is recommended by the European Fair Trade Association Surveillance Authority (Anonymous 2002). The fourth one is a non-pathogenic *E. coli* strain, proposed for testing antibiotics, antimicrobial preservatives and chemotherapeutic agents, and is used in the European Standard UNE-EN 1276 (Anonymous 1998) for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas.

The particular dilution employed in this work (having 86–93 mg l^{-1} active chlorine) was chosen on the basis of previous studies carried out in our laboratories (data not shown), designed to find the minimum concentration of NEW complying with the European Standard UNE-EN 1276 (Anonymous 1998), i.e. producing a reduction in more than 5 log CFU mg l^{-1} in all the evaluated strains in pure culture. In the present work as well, the populations of all the strains in pure culture were reduced by more than 5 log CFU mg l^{-1} within 5 min of exposure to NEW containing 89 mg l^{-1} active chlorine (444 mg l^{-1} before dilution). Moreover, these results are similar to those obtained by other authors using AEW to inactivate the same pathogens (Venkitanarayanan *et al.* 1999b; Kim *et al.* 2000a). This fact leads to conclude that it is the active chlorine content, rather than the low pH or high ORP as originally assumed (Kim *et al.* 2000b; Len *et al.* 2000), the main contributor to the bactericidal activity of electrolyzed water.

The main aim of this study was however to assess NEW effectiveness as a disinfectant for tomato surfaces. In this regard, initial populations of 5 log CFU sq.cm^{-1} on the surface of tomatoes were reduced to <1 log CFU sq.cm^{-1}. Moreover, no cells of any strain were detected by plating procedure in the NEW after treatment, suggesting that NEW could prevent cross-contamination of fresh produce and processing environments. In contrast, for deionized sterile water wash, an average count of 3 log CFU sq.cm^{-1} was detected on surface, and about 5 log CFU ml^{-1} still recovered from the wash solution.

As it is known to occur with AEW (Bari *et al.* 2003), the treatment with NEW also revealed to have a broad spectrum of action over the chosen pathogenic strains: their populations on tomato surface underwent similar reductions, without significant difference ($P \leq 0{\cdot}05$), after being rinsed during the same amount of time. Moreover, the surviving population of each pathogenic strain after a 60-s rinse in NEW showed no significant difference ($P \leq 0{\cdot}05$) with that observed after a 30-s wash. This fact leads to conclude that a 30-s treatment with 1 : 5 diluted NEW is enough for the disinfection of tomato surface.

The sensory evaluation has demonstrated that after washing tomatoes with NEW, no significant difference in taste, appearance or smell was detected by panellists. Hence, besides the efficacy to control *E. coli* (both pathogenic and nonpathogenic stains), *S. enteritidis* and *L. monocytogenes* on surfaces, the treatment is not expected to affect consumer acceptance of the product.

In relation with other disinfectants, AEW has shown to be more effective than ozonated water in sanitizing vegetables (Koseki *et al.* 2001), and similarly or more effective than chlorinated water (having the same pH, ORP and active chlorine values) in treating vegetables (Park *et al.* 2001; Kim *et al.* 2003) or pure culture of food-related pathogens (Kim *et al.* 2000b). The reductions obtained in this study using NEW are equal or superior to results obtained by washing different vegetables and surfaces with AEW of similar active chlorine content (Izumi 1999; Venkitanarayanan *et al.* 1999a; Koseki and Itoh 2001; Kim *et al.* 2003). These data suggest that NEW has a similar bactericidal efficacy to that of other agents, with the advantage of being a noncorrosive, safe and easy to handle option.

In summary, the findings of this study reveal that NEW is an effective method to significantly reduce the presence of pathogenic micro-organisms like *E. coli* O157:H7, *S. enteritidis* and *L. monocytogenes* on the surfaces of tomatoes, without affecting their organoleptic characteristics. This hints at its potential application for the decontamination of fresh produce contact surfaces.

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