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DECONTAMINATION EFFICACY OF NEUTRAL AND ACIDIC ELECTROLYZED WATER IN FRESH-CUT SALAD WASHING

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ABSTRACT

The possibility of washing fresh-cut vegetables with neutral (NEW) and acidic (AEW) electrolyzed water instead of highly chlorinated solution was investigated. The decontamination efficacy was tested *in vitro* against *Pseudomonas fluorescens*, which is a typical inhabitant of leafy vegetables. The antibacterial efficacy of NEW and AEW was highly dependent on pH solution. NEW and AEW that were adjusted to a pH analogous to that of salad (6.5) completely inactivate *P. fluorescens* at 30 mg/L free chlorine concentration. The same result was achieved by sodium hypochlorite aqueous solution at a much higher free chlorine concentration (150 mg/L). NEW and AEW at 30 mg/L free chlorine concentration also reduced total mesophilic count, *Pseudomonas* spp. and total coliforms of salad by *circa* 1 log cycle. Microbial loads of wastewater deriving from salad washing resulted below the detection limit.

PRACTICAL APPLICATIONS

Electrolyzed water can be exploited as a sanitizing washing agent in the fresh-cut industry allowing to replace conventional chlorination of washing water with highly concentrated NaOCl. Thus, it would be possible to guarantee an analogous decontamination efficacy and decrease the overall presence of potentially toxic chlorine compounds in wastewater. This would contribute to meet the global requirement of reducing the water footprint of industrial washing and reduce the use of harmful disinfection solutions.

INTRODUCTION

The increase in fresh-cut produce consumption over the last decades has gone along with the growing concerns about the foodborne illness associated to this produce. In fact, fresh-cut vegetable, and leafy ones in particular, are among the products most frequently implicated in foodborne outbreaks (EFSA 2013; FDA 2013). To date, washing is the only processing step that can reduce the microbial load of the product (Artes *et al.* 2009). The turbulent flow of wash water promotes the mechanical removal of microorganisms from vegetable surface, leading to a reduction in microflora of *circa* 1 log unit (Allende *et al.* 2008). As a consequence, the water within the washing tank is characterized by high load in microorganisms, unless a proper disinfection strategy is in place.

To reduce wash water microbial load and thus avoid cross-contamination associated with the reuse of water, the latter is added with disinfection chemicals. Chlorine, especially in the form of NaOCl, is the most exploited water disinfectant. It is used at levels of 120–200 mg/L of free chlorine and pH values of 6.0–7.5 to ensure the presence of hypochlorous acid (HOCl) and minimize equipment corrosion (Parish *et al.* 2003). However, the use of high chlorine concentrations may cause the generation of irritating chlorine gas and may lead to the formation of disinfection by-product (DBPs). Among the latter, trihalomethanes and haloacetic acids have been recognized as carcinogenic and/or mutagenic compounds (Hua and Reckhow 2007; Legay *et al.* 2010). Because of the possible formation of DBPs, the use of chlorine in fresh-cut produce washing is not allowed in some European countries such as Germany, the Netherlands, Denmark, Belgium and Switzerland (Van Haute *et al.* 2013). Thus, it is crucial to identify efficacious water disinfection strategies other than those based on highly concentrated NaOCl in order to reduce or avoid the use of chlorinated compounds (Siroli *et al.* 2015).

Currently, electrolyzed water (EW) is gaining importance as a sanitizer in the food industry. It is usually generated by electrolysis of a NaCl aqueous solution in a chamber, within which the electrodes are separated or not by a semipermeable membrane. When membrane separation is performed, two types of water are produced. At the anode side, an acidic electrolyzed water (AEW) solution with pH value of 2.3–2.7, containing free chlorine (HOCl and OCl^-) and having high oxidation reduction potential (ORP; >1100 mV), is produced. On the contrary, an alkaline EW solution (pH 11–11.7) with low ORP (−800 to −900 mV) and absence of chlorine is generated at the cathode side (Hsu 2005). In the case of electrolysis in the absence of a separating membrane, a neutral electrolyzed water (NEW) solution with pH in the range of 8.0–8.5, ORP of 650–750 mV and free chlorine content is produced (Al-Haq *et al.* 2005).

Several studies attributed to AEW and NEW a high antimicrobial efficacy *in vitro* (Issa-Zacharia *et al.* 2010; Rahman *et al.* 2012). The enhanced bactericidal effect compared with highly concentrated chlorinated solutions obtained by addition of NaOCl could be attributable to the presence in EW of reactive oxygen species (ROS) such as $\cdot OH^-$, O_3 and H_2O_2 (Jeong *et al.* 2006; Hao *et al.* 2012). Because of the intense antimicrobial effect, AEW and NEW have been proposed for decontamination of working surfaces and equipments (Park *et al.* 2002; Ayebah *et al.* 2006) as well as of food products including meat, seafood, fruit and vegetables (Park *et al.* 2009; Pangloli and Hung 2011; Xie *et al.* 2012; Rahman *et al.* 2013). Particularly interesting seems the application of electrolyzed solutions as washing agents of fresh-cut produce. To this regard, Forghani and Oh (2013) demonstrated that EW, even at low free chlorine concentration of 21–22 mg/L, allowed inactivation of microorganisms inoculated in leafy vegetables. In addition, Tomás-Callejas *et al.* (2011) not only proved that EW was as effective as the conventional disinfection with NaOCl in salad decontamination but also indicated that it did not affect the quality of the fresh-cut product. Furthermore, some studies investigated the effect of EW on microbial contamination of salad wash water. The latter was efficaciously decontaminated allowing to maintain the microbiological and physiochemical quality of wash water (López-Gálvez *et al.* 2012; Gómez-López *et al.* 2014).

However, available literature is limited to the study of the antimicrobial activity of electrolyzed solutions having a given free chlorine concentration with no clear indication on the optimal physicochemical properties (i.e., free chlorine concentration, pH and ORP) allowing to obtain the same bactericidal effect of concentrated NaOCl aqueous solution. In addition, there is a lack of information about the effect of EW on both wash water and salad.

The aim of this study was to determine which are the physicochemical properties that allow NEW and AEW to replace sodium hypochlorite as a disinfectant in fresh-cut salad washing. To this purpose, NEW and AEW at increasing concentrations of free chlorine and different pH and ORP values were prepared. The germicidal efficacy of electrolyzed solutions was firstly evaluated on pure culture of *Pseudomonas fluorescens* in order to identify the minimum concentration of free chlorine for bacteria inactivation below the detection limit. Subsequently, NEW and AEW were used for salad washing. The decontamination efficacy was tested in both wash water and salad.

MATERIALS AND METHODS

Preparation of Treatment Solutions

EW solutions were generated using an Envirolyte Demonstration Unit (mod. Dem-30, Envirolyte Industries International Ltd., Tallin, Estonia) provided with two platinum–iridium coated titanium electrodes separated by an yttria-zirconium membrane. The EW solutions were produced at room temperature using a continuous supply of NaCl (Carlo Erba, Milano, Italy) aqueous solution at increasing concentration up to 3.4 g/L. NEW solutions were collected after electrolysis without the use of the separation membrane. When membrane separation was performed, AEW and alkaline solutions were collected at the anode and cathode side, respectively. EW pH adjustment was carried out with 0.1 M HCl (Sigma Aldrich, Milano, Italy) and 0.1 M NaOH (Sigma Aldrich) for NEW and AEW, respectively.

NaOCl aqueous solutions (Titolchimica, Rovigo, Italia) at increasing concentration of free chlorine and pH adjusted to 6.5 with HCl (0.1 M, Sigma Aldrich) were also prepared as control.

Bacteria Strain and Growth Conditions

The microorganism used in this study was *P. fluorescens* L22 obtained from the Department of Food Science Collection, University of Udine (Italy). The strain was maintained at −80C in tryptone soya broth (TSB; Oxoid, Basingstoke, UK) with 30% glycerol added as a cryogenic agent. The strain was kept in TSB at 37C for 48 h and subsequently spread on tryptone soya agar (Oxoid) and incubated under the same conditions. The strain was cultured in 5 mL of TSB at 37C for 24 h, collected by centrifugation at 13,000 rpm for 10 min at 4C (Beckman, Avanti J-25, Palo Alto, CA) and washed three times with maximum recovery diluent (MRD; Oxoid). Final pellets were suspended in MRD and used for *in vitro* tests. *Pseudomonas* agar base (PSA; Oxoid) plates were used for enumeration of *P. fluorescens*. Plates were incubated for 48 h at 30C.

In Vitro Inactivation of *P. fluorescens*

For *in vitro* inactivation of *P. fluorescens*, 1 mL of bacterial suspension was added to 9 mL of NEW, AEW or NaOCl solutions in a sterile test tube and vortexed (Fisher Scientific, Illkirk, France). The inactivation treatment was carried out for 2 min at room temperature. Inactivation experiments were stopped by transferring 1 mL of each treated sample to a sterile tube containing 9 mL of neutralizing 0.5% (w/v) sodium thiosulfate ($Na_2S_2O_3$; Sigma Aldrich, Milano, Italy) aqueous solution. After neutralization, samples were then used for microbial enumeration.

Salad Washing

Lamb's lettuce was provided by a local farm, stored at 6C and processed within 1 day. Lamb's lettuce was washed in the washing solutions at 8C for 2 min with 1:10, w/v salad/water ratio. Lamb's lettuce leaves were separated from wash water and centrifuged (mod. ACX01, Moulinex, Ecully, France) for 1 min. Water drained from leaves by centrifugation was added to the previously collected wash water. Water and lettuce samples were immediately used for analysis.

Temperature, pH, ORP and Chlorine Determinations

Temperature was measured by a thermocouple probe (Checktemp1, Hanna Instruments, Inc., Woonsocket, RI).

pH and ORP were measured with a Crison 50 12T electrode (Hach Lange, Barcelona, Spain) and a InLab redox-combined electrode (Schwerzenbach, Switzerland), respectively, connected to a pH meter (PH301, Hanna Instruments, Smithfield, RI).

Free chlorine was determined by a HI38020 kit based on the use of N,N-diethyl-p-phenylenediamine (Hanna Instruments Inc., Salaj, Romania).

HOCl and OCl^- concentrations were assayed spectrophotometrically at 234 and 292 nm, respectively, using a UV-vis spectrophotometer (Shimadzu UV-2501PC, Shimadzu Corporation, Kyoto, Japan) and determined by Beer's law equation (Len *et al.* 2000).

Microbiological Analysis

Washing solutions and salad were sampled for microbial plate counts. In particular, before plating, 10 g of salad samples was added to 90 mL of MRD (Oxoid) and homogenized for 2 min in a Stomacher Lab-Blender 400 (PBI International, Milano, Italy). Serial dilutions of each suspension were made in MRD (Oxoid) and appropriate aliquots (0.1 or 1 mL) were spread on agar plates. Plate count agar (Oxoid) and PSA (Oxoid) were used for enumeration of total mesophilic bacteria and *Pseudomonas* spp., respectively; plates were incubated at 30C for 48 h. Coli ID (BioMerieux, Mercy L'Etoile, France) was used for enumeration of total coliforms; plates were incubated at 37C for 24 h. Detection limit for total mesophilic bacteria and *Pseudomonas* was <100 cfu/mL and <100 cfu/g for washing solution and salad, respectively. In the case of coliforms, the detection limit was <10 cfu/mL and <10 cfu/g for washing solution and salad, respectively.

Statistical Analysis

At least duplicate analyses were performed on at least duplicated samples. Results are reported as mean value ± standard deviation. Linear regression analyses was performed using Microsoft Office Excel 2007. Analyses of variance were performed with significance level set to $P < 0.05$ (Statistica for Windows, ver. 5.1, Statsoft Inc. Tulsa, OK, 1997). The Tukey's procedure was used to test for differences between means.

RESULTS AND DISCUSSION

Physicochemical and Antimicrobial Properties of Electrolyzed Solutions

Aqueous solutions containing increasing concentration of sodium chloride were electrolyzed to obtain NEW solutions that were analyzed for free chlorine concentration, pH and oxidizing reduction potential (ORP) (Table 1). Free chlorine concentration progressively increased with the initial

TABLE 1. PHYSICOCHEMICAL PROPERTIES OF NEUTRAL ELECTROLYZED WATER (NEW) OBTAINED BY ELECTROLYSIS OF NaCl SOLUTIONS AT INCREASING CONCENTRATION

NaCl (g/L)	Free chlorine (mg/L)	pH	ORP (mV)
1.00	16 ± 1^{g}	8.0 ± 0.0^{d}	689 ± 13^{a}
1.25	31 ± 1^{f}	8.2 ± 0.0^{c}	666 ± 27^{a}
1.60	43 ± 4^{e}	8.4 ± 0.0^{b}	656 ± 20^{a}
1.80	64 ± 4^{d}	8.4 ± 0.0^{b}	642 ± 12^{a}
2.00	81 ± 5^{c}	$8.4 \pm 0.1^{a,b}$	637 ± 35^{a}
2.80	123 ± 7^{b}	$8.5 \pm 0.1^{a,b}$	650 ± 26^{a}
3.30	152 ± 5^{a}	8.5 ± 0.0^{a}	646 ± 14^{a}

Means with same letter in each column are not significantly different ($P \geq 0.05$).
ORP, oxidation reduction potential.

sodium chloride concentration. The latter slightly affected the pH of the electrolyzed solutions, which ranged from 8.0 to 8.5. By contrast, ORP resulted quite high, independently on the initial NaCl concentration, in accordance with literature data (Abadias *et al.* 2008).

Analogous electrolyzed solutions were produced by separating the acidic and alkaline water during electrolysis by a separation membrane. The alkaline water presented high pH (11.7 ± 0.3), low ORP (−882 ± 23 mV) and absence of chlorine compounds. Based on these properties and not exerting antimicrobial effects, the alkaline water was not considered in this study and the attention was focused on the AEW that contained high levels of antimicrobial chlorine compounds. Table 2 shows the physicochemical properties of AEW obtained by electrolysis of NaCl solutions at increasing concentration. At the same initial concentration of sodium chloride, AEW showed chlorine concentration (Table 2) not significantly different ($P \geq 0.05$) from that observed in the NEW (Table 1). However, the AEW was characterized by much lower pH and much higher ORP. As shown in Table 2, even in this case, pH and ORP values were slightly affected by NaCl concentration.

Based on these properties, both NEW and AEW solutions could be used as washing solutions to avoid cross-contamination during fruit and vegetable washing in the fresh-cut industry. However, their different physicochemical properties could be associated with diverse antimicrobial activity. In order to evaluate the germicidal effect of electrolyzed solutions, *in vitro* tests against *P. fluorescens* were performed. The latter belongs to a genus particularly diffused in leafy vegetables and was chosen as a representative microorganism responsible for hygienic quality decay (Ongeng *et al.* 2006; Manzocco *et al.* 2010; Schwaiger *et al.* 2011). The survival of *P. fluorescens* at 9 log concentration was tested in the presence of NEW and AEW solutions containing increasing concentration of free chlorine. Figure 1 reports the effect of different solutions on the microbial load of *P. fluorescens.*

TABLE 2. PHYSICOCHEMICAL PROPERTIES OF ACIDIC ELECTROLYZED WATER (AEW) OBTAINED BY ELECTROLYSIS OF NaCl SOLUTIONS AT INCREASING CONCENTRATION

NaCl (g/L)	Free chlorine (mg/L)	pH	ORP (mV)
1.00	14 ± 2[g]	2.4 ± 0.0[a]	1,177 ± 3[c]
1.25	29 ± 2[f]	2.4 ± 0.0[a]	1,181 ± 2[c]
1.60	42 ± 4[e]	2.3 ± 0.0[b]	1,183 ± 3[c]
1.80	61 ± 5[d]	2.3 ± 0.0[b]	1,183 ± 5[b,c]
2.00	78 ± 4[c]	2.3 ± 0.0[b]	1,185 ± 2[b,c]
2.80	115 ± 7[b]	2.3 ± 0.0[b]	1,188 ± 1[b]
3.30	146 ± 5[a]	2.3 ± 0.0[b]	1,193 ± 3[a]

Means with same letter in each column are not significantly different ($P \geq 0.05$).
ORP, oxidation reduction potential.

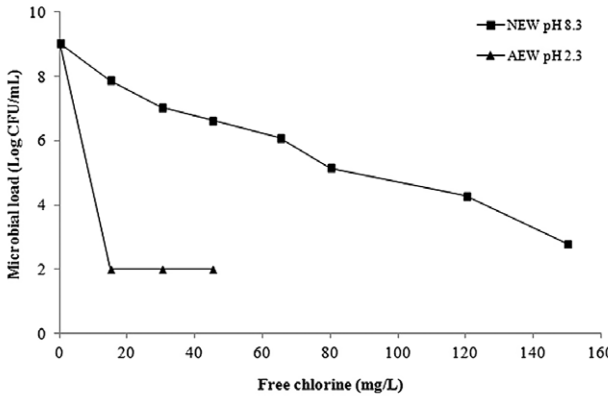

FIG. 1. MICROBIAL LOAD OF *PSEUDOMONAS FLUORESCENS* AS A FUNCTION OF FREE CHLORINE CONCENTRATION OF NEUTRAL (NEW) AND ACIDIC (AEW) ELECTROLYZED WATER

By increasing the concentration of free chlorine, NEW solutions (Table 1) exerted increasing bactericidal effect on *P. fluorescens*, reaching a microbial load reduction of *circa* 7 log at 150 mg/L free chlorine. On the contrary, AEW solutions (Table 2) completely inactivated the microorganism at the lowest free chlorine concentration tested here (15 mg/L). This result can be attributed to the well-known antimicrobial effect of acid pH on cell membranes as well as to the high ORP value (1,177 ± 3 mV; Table 2). Data shown in Fig. 1 are in agreement with those reported by Issa-Zacharia *et al.* (2010) on microorganisms other than *P. fluorescens*. These authors observed almost 6 log reductions of *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* spp. in the presence of EW with 1,140 mV ORP, pH 2.6 and 25 mg/L free chlorine. By contrast, at the same free chlorine content, the increase in pH exerted a lower germicidal effect (Issa-Zacharia *et al.* 2010).

Effect of pH Adjustment on Physicochemical and Antimicrobial Properties of Electrolyzed Solutions

Literature data suggest that the germicidal efficacy of chlorinated water solutions is highly affected by pH adjustment (Len *et al.* 2000; Hricova *et al.* 2008). The original pH of aqueous solutions of sodium hypochlorite (NaOCl) intended for vegetable washing is about 8, depending on the concentration. The solution pH is generally adjusted to pH ranging from 4 to 6.5 to maximize the germicidal effect (Len *et al.* 2000; Park *et al.* 2004). Under these conditions, the equilibrium among the different chlorine species (gaseous chlorine, hypochlorous acid and hypochlorite ion) largely favors the prevalence of hypochlorous acid, which is the most active antimicrobial form (Kim *et al.* 2000; Abadias *et al.* 2008). A similar equilibrium is also reached in

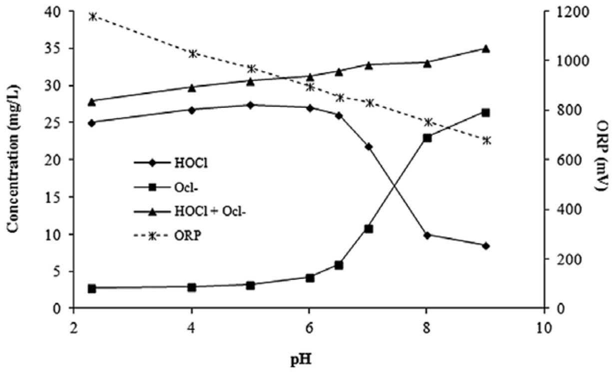

FIG. 2. CONCENTRATION OF HOC1, OC1, THEIR SUM AND OXIDATION REDUCTION POTENTIAL IN ACIDIC ELECTROLYZED WATER (AEW) OBTAINED BY ELECTROLYSIS OF 1.25 G/L NaCL AQUEOUS SOLUTION AND ADJUSTED TO INCREASING pH

electrolyzed solutions. To this regard, Fig. 2 shows the concentration of HOCl, OCl^- and their sum in AEW obtained by electrolysis of 1.25 g/L NaCl aqueous solution (initial pH 2.4 ± 0.0; Table 2) adjusted at different pH up to 9.0.

At alkaline pH, the concentration of HOCl was lower than that of OCl^-, while opposite equilibrium of these species was observed at acid pH making the solution highly germicidal (Len *et al.* 2000; Hao *et al.* 2012). In fact, HOCl was demonstrated to be 20 times more effective against microorganisms than OCl^- (White 1999). Below pH 4, HOCl is known to dissociate and form chlorine gas, which is highly volatile and toxic (Shigeto *et al.* 2000). Similar results were also achieved by adjusting the pH of NEW solutions (data not shown), reflecting the general behavior of chlorinated aqueous solutions that depends solely on pH. Similarly, also the chemical equilibrium of electrolyzed solutions only depends on pH and is not affected by the initial concentration of NaCl before electrolysis. However, the latter affects the overall amount of chlorine species in AEW and NEW solutions and, consequently, their germicidal efficacy. In addition, the modification of equilibrium among chlorine species upon pH adjustment strongly modified ORP value (Fig. 2). In particular, the increase in pH was associated with a decrease in ORP and thus potentially in the antimicrobial activity (Fig. 2).

Based on these considerations, the pH of NEW and AEW solutions (Tables 1 and 2) could be adjusted to slightly acid values to maximize the antimicrobial capacity. However, the physicochemical properties of electrolyzed solutions should not impair product quality. To this regard, optimal pH of washing solutions should approach that of the vegetable. Because salad is characterized by a pH of *circa* 6.5, its washing should be performed at such a value to maintain product quality (Rico *et al.* 2008; Tomás-Callejas *et al.* 2011). NEW and AEW solutions having increasing free chlorine concentration were thus adjusted to pH 6.5. Table 3 shows that the modification of the equilibrium among chlorine species was allowed to obtain NEW and AEW with similar ORP. In particular, pH adjustment increased the ORP values of NEW by *circa* 150 mV as compared with the non-pH-adjusted NEW (Table 1). It can be hypothesized that the increase in NEW oxidative capacity could improve its germicidal activity. On the contrary, by increasing the pH of AEW a decrease in ORP of *circa* 300 mV was observed, probably leading to a decrease in the germicidal activity solution.

To verify these hypothesis, the effect of AEW and NEW solutions adjusted at pH 6.5 on the microbial load of *P. fluorescens* was tested (Fig. 3). An aqueous solution containing increasing amount of free chlorine compounds but obtained by adding NaOCl was also considered as control. As expected, by increasing the concentration of free chlorine, the control NaOCl solution showed a higher bactericidal effect on *P. fluorescens*. In particular, the microbial load was reduced below the detection limit when the free chlorine concentration of NaOCl solutions approached

TABLE 3. ORP (mV) OF NEUTRAL (NEW) AND ACIDIC (AEW) ELECTROLYZED WATER CONTAINING INCREASING FREE CHLORINE CONCENTRATION AND ADJUSTED TO pH 6.5

Free chlorine (mg/L)	NEW	AEW
15 ± 2	812 ± 11[b]	865 ± 13[a]
30 ± 2	805 ± 9[b]	862 ± 17[a]
42 ± 3	789 ± 12[b]	879 ± 6[a]
63 ± 4	795 ± 14[b]	881 ± 9[a]
80 ± 4	807 ± 4[b]	878 ± 11[a]
119 ± 7	803 ± 7[b]	886 ± 8[a]
151 ± 6	806 ± 15[b]	889 ± 12[a]

Means with same letter are not significantly different ($P \geq 0.05$).
ORP, oxidation reduction potential.

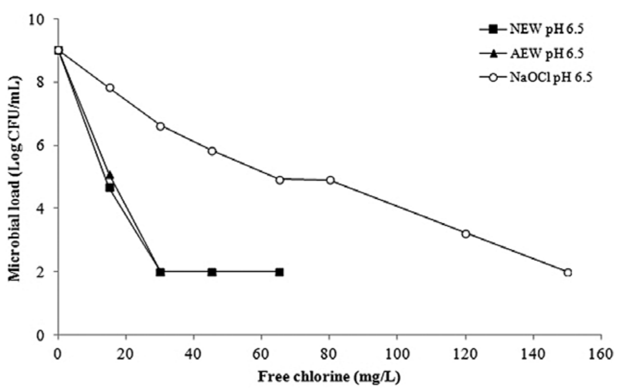

FIG. 3. MICROBIAL LOAD OF *PSEUDOMONAS FLUORESCENS* AS A FUNCTION OF FREE CHLORINE CONCENTRATION OF NEUTRAL (NEW) AND ACIDIC (AEW) ELECTROLYZED WATER SOLUTION AT pH ADJUSTED TO 6.5

TABLE 4. NATIVE MICROFLORA OF LAMB'S LETTUCE WASHED WITH NEUTRAL (NEW) OR ACIDIC (AEW) ELECTROLYZED WATER

Washing	Free chlorine concentration (mg/L)	Microbial load (log cfu/g)		
		Total mesophilic count	*Pseudomonas* spp.	Total coliforms
None	–	7.6 ± 0.1^{a}	7.4 ± 0.2^{a}	3.3 ± 0.1^{a}
Deionized water	0	6.3 ± 0.3^{b}	5.9 ± 0.2^{b}	2.0 ± 0.1^{b}
NaOCl	150 ± 4	6.1 ± 0.4^{b}	5.9 ± 0.2^{b}	1.9 ± 0.3^{b}
NEW	30 ± 2	6.5 ± 0.1^{b}	6.4 ± 0.3^{b}	2.4 ± 0.2^{b}
AEW	30 ± 2	6.5 ± 0.2^{b}	6.0 ± 0.3^{b}	2.2 ± 0.3^{b}

Means with same letter in each column are not significantly different ($P \geq 0.05$). Samples washed with deionized or NaOCl solution are shown as control.

150 mg/L. It is noteworthy that this concentration is conventionally used in industrial salad washing (Beuchat 1998). In the case of the electrolyzed solutions, the inactivation of *P. fluorescens* below the detection limit was achieved at 30 mg/L free chlorine. At the same concentration and pH, the higher germicidal activity of electrolyzed solutions as compared with NaOCl solution could be attributed to their higher ORP. The higher bactericidal effect of electrolyzed solutions could be also due to the well-known presence of ROS such as O_3 and $\cdot OH^-$ (Jeong *et al.* 2006).

The complete inactivation of *P. fluorescens* by EW was also reported by Ongeng *et al.* (2006) at a chlorine concentration of *circa* 4 mg/L. In this study, no indication on the pH and ORP of the solution was provided but the complete inactivation of *P. fluorescens* was achieved upon 45 min of contact with the EW solution. By contrast, in the present experiment, only 2 min of contact was allowed.

Antimicrobial Efficacy of Neutral and Acidic Electrolyzed Solutions in Fresh-Cut Salad Washing

To verify the applicability of electrolyzed solutions in vegetable washing, NEW and AEW at pH 6.5 and containing the minimum free chlorine concentration that were able to guarantee the inactivation of *P. fluorescens* below the detection limit (30 mg/L; Fig. 3) were used to perform lamb's lettuce washing. Washing was also carried out using a NaOCl solution containing 150 mg/L free chlorine (pH 6.5 ± 0.1 and ORP 724 ± 43 mV). This concentration was chosen as *P. fluorescens* was allowed to be reduced below the detection limit (Fig. 3). The decontamination efficacy was also evaluated in the absence of chlorine compounds by washing salad with deionized water (pH 6.8 ± 0.3 and ORP 375 ± 32 mV).

Unwashed salad was confirmed to be characterized by a microbial population greatly represented by *Pseudomonas* spp. (Table 4). The presence of total coliforms, which are typical inhabitants of fresh-cut plants deriving from soil and manure contamination, was also detected. Table 4 shows that, independently on the washing solution used and their free chlorine concentration, *circa* 1.5 log reductions in total mesophilic count and *Pseudomonas* spp. were achieved. Similar results were obtained for total coliforms with *circa* 1 log reduction.

This antimicrobial efficacy is in accordance with literature data on leafy vegetable washing and can be attributed to the ability of water turbulent flow to mechanically remove the contaminating bacteria at the product surface (Allende *et al.* 2008). Therefore, no germicidal effect on the internalized microorganism in vegetable tissue can be achieved upon washing (Hou *et al.* 2013). For this reason, the overall antimicrobial effect of washing on salad difficulty exceeds 1.5–2 log reductions. However, it can be hypothesized that the decontamination efficacy of electrolyzed solutions could be mainly observed in wash water. Based on these considerations, microbial analyses were also performed on the wastewater recovered after lettuce washing with the solutions considered here (Table 5).

As expected, wastewater derived from salad washing in deionized water resulted to be highly contaminated with counts approaching those of unwashed salad (Table 4). Microbial load also resulted in the same magnitude range reported in the literature for wastewater from industrial washing of different vegetables (Selma *et al.* 2008; Barrera *et al.* 2012). NaOCl solution containing 150 mg/L of free chlorine was able to reduce the microbial count of wastewater below the detection limits. Analogous results were also obtained with NEW and AEW solutions at pH 6.5. However, the latter was characterized by a free chlorine content of 30 mg/L, which is five times lower than that of the NaOCl solution. It can be inferred that electrolyzed solutions with low free chlorine concentration could be efficiently employed to perform vegetable washing. Their application should necessarily guarantee that wash water maintains its germicidal activity during multiple washing cycles. This could be achieved by maintaining a constant 30 mg/L concentration of free chlorine compounds in wash water by continuous enrichment with newly generated EW.

TABLE 5. NATIVE MICROFLORA OF WASTEWATER RECOVERED AFTER LAMB'S LETTUCE WASHING WITH NEUTRAL (NEW) OR ACIDIC (AEW) ELECTROLYZED WATER

Washing	Free chlorine concentration (mg/L)	Microbial load (log cfu/mL)		
		Total mesophilic count	*Pseudomonas* spp.	Total coliforms
Deionized water	0	6.5 ± 0.4	5.5 ± 0.3	2.6 ± 0.2
NaOCl	150 ± 4	<DL	<DL	<DL
NEW	30 ± 2	<DL	<DL	<DL
AEW	30 ± 2	<DL	<DL	<DL

DL, detection limit (<2 log cfu/mL for total mesophilic bacteria and *Pseudomonas* spp.; <1 log cfu/mL for total coliforms). Wastewater from washing with deionized water or NaOCl solution are shown as control.

CONCLUSION

Results demonstrate that EW can be exploited as a sanitizing washing agent in the fresh-cut industry. The use of electrolyzed solutions would allow to replace conventional chlorination of washing water with highly concentrated NaOCl, guaranteeing an analogous decontamination efficacy and decreasing the overall presence of potentially toxic chlorine compounds in wastewater. This would contribute to meet the global requirement of reducing the water footprint of industrial washing. In addition, the possibility of producing *in situ* germicidal wash water by electrolysis of NaCl aqueous solutions would avoid the presence of harmful NaOCl within the food industry, limiting the health risks for operators as well as for product contamination.

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