



DECONTAMINATION EFFECT OF ELECTROLYZED WATER WASHING ON FRUITS AND VEGETABLES

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Review



ABSTRACT

The use of electrolyzed water in the washing of fruits and vegetables is a promising alternative treatment to chlorine washing. Electrolyzed water washing, is safer, healthier, reduces cleaning times, and is ready to handle. In recent years, food poisoning outbreaks which are caused by bacteria with acid tolerance response in fruits and vegetables has increased. In addition, pathogen produce cases and outbreaks linked to fresh fruits and vegetables, such as cantaloupes, strawberries, fruit salads, spinach, lettuce, celery, and tomatoes has been encountered. Nowadays, the necessity of effective and healthy decontamination processes has gained more importance. The aim of this review is to offer a complete view about electrolyzed water, its classifications and applications. Decontamination results of extant literature of electrolyzed water were also presented. Also, the effects and results of electrolyzed water decontamination on the microbial counts of fresh fruits and vegetables compared with different sanitizing agents have been summarized.

Keywords: electrolyzed water, microbial reduction, fruits and vegetables

INTRODUCTION

There has been an increased concern about food safety regarding acute food borne diseases, infections, and intoxications. Despite the progresses in food science, food processing, and food hygiene, consumer awareness, food borne illnesses mediated by pathogenic microorganisms are still widespread and represent a significant threat to public health all over the world (Huang *et al.*, 2008; Issa-Zacharia *et al.*, 2011). In addition, food poisoning outbreaks, which are caused by bacteria with acid tolerance response in fruits and vegetables, has shown an increasing trend (Weissinger *et al.*, 2000; CDC, 2009). Normally, the pH of fruits and vegetables is below the level that generally favors bacterial growth, although it is known that acid tolerance pathogens produce cases and outbreaks linked to fresh fruits and vegetables such as cantaloupes, strawberries, fruit salads, spinach, lettuce, celery, and tomatoes has increased. Over the past two decades, fruits and vegetables have repeatedly become a source of foodborne illnesses. The different pathogens most frequently linked to fruit and vegetable produce-related outbreaks generally include bacteria such as *Escherichia coli* O157: H7, *Salmonella* spp., and *Listeria* spp. which are a public health concern (CDC, 2006; Nguyen-The, 2012; Olaimat & Holley, 2012).

In order to obtain the desired microbiological quality in the food industry, the primary and the most important criterion is to achieve minimizing the number of microorganisms which contaminate the raw food material through different sources. This step should be done efficiently without decreasing the nutritional value of the food and damage to the environment. Decontamination of fresh fruits and vegetables is an important unsolved technological problem. The necessity for an effective wash water decontamination process in the raw material section of the fruit and vegetable industry is a very critical step. In fruit and vegetable cultivation, the possible contamination sources are seed, soil, irrigation water, animals, manure, and the use of sewage sludge. The washing methods can reduce the microbial load of the product. Thus, antimicrobial efficiency of the washing step could be thought as the assurance of the microbiological quality of the final product. Besides removing the microorganisms efficiently with washing, the selected method ought to be easily adapted to commercial lines, and should be simple and cheap (Sivapalasingam *et al.*, 2004; Ersus-Bilek & Turantas, 2013; Turantaş *et al.*, 2015). Several antimicrobial agents such as sodium hypochlorite, hydrogen peroxide, ethanol, organic acids, and ozone have been used as sanitizers in the food industry, besides their potential toxicity, they have proved incapable of completely removing or inactivating pathogens in food

supplies. Currently, chlorine and chlorine derivatives regarded as cheap, and effective, are widely used sanitizers for reducing microbial contamination in the fresh-cut food industry. During the process, the chlorine solution loses its activity with exposure to air, light, metals, and its vapors may cause irritation to the skin and the respiratory tract (Hostynek *et al.*, 1990; Dychdala, 2001). The by-products, trihalomethanes (THMs), chloroform, and chlorophenols carry a health risk because they are potentially mutagenic and have been classified by the US Environmental Protection Agency (EPA) as possible human carcinogens (Ukuku & Fett, 2006; Ölmez & Kretzschmar, 2009; Cho *et al.*, 2010). Many regulations and guidelines have been applied to the food industry to stimulate the minimum activated chlorine concentration (ACC) in water streams (Sharma & Demirci, 2003; Issa-Zacharia *et al.*, 2010; Zhang *et al.*, 2011).

The electrolyzed oxidizing water (EOW), with low ACC is one of the potential alternatives to sodium hypochlorite usage with an environmentally friendly broad spectrum microbial decontamination in the food industry, so there is no need for the handling and storing of potentially dangerous chemicals (Phuvasate & Su, 2010; Whangchai *et al.*, 2010; Sapers, 2014). EOW is created with the electrolysis of diluted sodium, potassium, and magnesium chloride solutions in an electrolysis chamber, having free chlorine in low concentration (low ACC) as a disinfection agent. EOW has the same or higher antimicrobial activity as a hypochlorite solution, even though it has less available chlorine than hypochlorite, it results greater effectiveness due to the continual production of available chlorine in the flow through the electrolysis chamber without hyperchlorination (Koseki & Itoh, 2001; Kiura *et al.*, 2002; Al-Haq & Gómez-López, 2012). Especially, it has been reported that slightly acidic electrolyzed (SAEW) has some important advantages such as low ACC and high stability compare to the other antimicrobial agents. Because of these advantages, there has been growing interest in the use of SAEW as an alternative decontamination method. Hence, this review will provide an overview about EOW, its classifications and importance on treatments and decontamination effects of it as an alternative sanitizing agent to different chemicals especially chlorine in the fruit and vegetables decontamination processes. It defines the efficacy and properties of different kinds of EOW as a decontaminant and then focuses on the effect of the microbial counts in fruits and vegetables compared with different sanitizing agents.

PRODUCTION AND CLASSIFICATION OF ELECTROLYZED WATER

EOW is generated in a cell containing inert positively charged and negatively charged electrodes separated by a septum (it is called cationic membrane or diaphragm-Morita et al., 2000; Rivera, 2005). The schematic illustration of acidic, basic (alkaline), and slightly acidic (low concentration) electrolyzed water generating equipment and the resulting compound during electrolysis are shown in Fig 1A and 1B, respectively. The current in the electrolyzing chamber and voltage between the electrodes are approximately set at 8.10 amperes and 9.10 volts, respectively. A saturated NaCl (or KCl/MgCl₂) solution and tap water are passed through this chamber. When a current is passed through this electrolysial chamber, the solution is allowed to dissociate into the two streams of acidic and alkaline EOW which may be collected from their respective outlets:

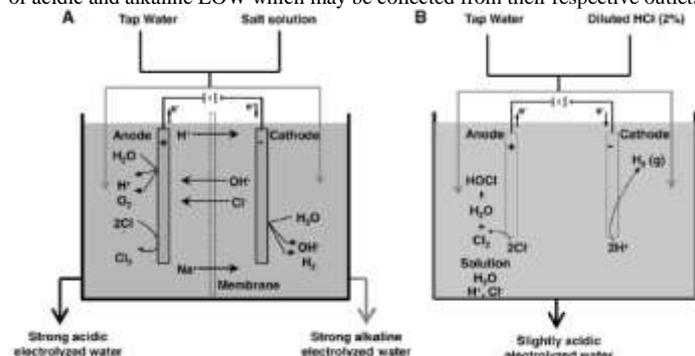


Figure 1 Schematic diagram of electrolyzed water and produced compounds during electrolysis (permission with Issa-Zacharia et al., 2010)

Acidic electrolyzed water (AEW, AcEW or strong acidic electrolyzed water –StAEW-)

An electrolyzed acid solution from the anode stream (pH <2.7, oxidation-reduction potential-ORP, 950-1180 mV, high dissolved oxygen, and chlorine base reactants -also known as available chlorine content - ACC) at 10 to 90 mg/L and contains hypochlorous acid (HOCl), OCl⁻, Cl₂, OH⁻, and O₃, which has a strong antimicrobial effect (Park et al., 2001; Krasakoopt & Bhandari, 2011; Teixeira & Rodrigues, 2014). For instance, AEW has been reported to have a strong bactericidal effect on most pathogenic bacteria such as *Escherichia coli* O157:H7, *Salmonella enteritidis*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, *Enterobacter aerogenes*, and *Campylobacter jejuni* (2-6 log unit reductions) which are important for food safety (Izumi, 1999; Al-Haq et al., 2001; Kim et al., 2001). In addition to this strong antimicrobial effect, AEW has some disadvantages such as corrosion of the processing equipment and is dangerous to human health such as irritation of the personnel's skin and consequently limits its practical application (Len et al., 2002; Koseki et al., 2003; Park et al., 2004). Primarily, it must be emphasized that the utilization of AEW has limited potential for long-term applications because of its strong acidity (pH<2.7), the antimicrobial effect of it can be rapidly decreased in time due to volatilization of dissolved Cl₂ gas and its negative impacts on human health and the environment. Despite not having any negative effect on the sensorial stability of products treated with AEW during refrigerated storage, some researchers mentioned that AEW can also have a deleterious effect such as loss of aroma, undesirable chemical burns, defects on the surface of leafy vegetables, browning of leaves, and electrolyte leakage caused by oxidation for some vegetables (Wang et al., 2004; Al-Haq et al., 2005; Rahman et al., 2010a).

Neutral (NEW), near-neutral (NNEW), or slightly acidic electrolyzed water (SAEW)

Due to its neutral pH, the neutral electrolyzed water (NEW) does not exhibit as aggressively as AEW to the corrosion of processing equipment or irritation of hands, and is more stable for chlorine loss (Abadias et al., 2008). NEW or SAEW (pH 5.0-6.5, ORP 800-900 mV, and ACC 10-30 mg/L) mainly contains biocidal reagents such as HOCl, ClO⁻, HO₂, and O₂ because of advantages over that of AEW, it was mostly used as disinfectants for foods, processing equipments, utensils and food contact surfaces (Al-Haq et al., 2002; Kim et al., 2003; Artés et al., 2009). SAEW has been an authorized food additive in Japan since 2002 because of its proven biological safety and antimicrobial effect even at low ACC of 10-30 mg/L (Suzuki et al., 2005; Issa-Zacharia et al., 2011). For instance, it was determined that NEW (or SAEW) was effective as a disinfectant for fresh-cut vegetables without causing discoloration, any detrimental effect of the tissue pH, surface colour, or general appearance of food products by different researchers (Izumi, 1999; Tomás-Callejas et al., 2011; Zhang et al., 2011). SAEW (NEW or low concentration EW) which has a stable and preferable microbiocidal effect than AEW, it may be related to the fact that dissolved chlorine does not decrease as much as AEW in time. The effective form of chlorine in SAEW is almost the HOCl having strong antimicrobial activity (Yoshifumi, 2003; Huang et al., 2008; Koide et al., 2009).

Basic electrolyzed water (BEW, ER water or AIEW)

An electrolyzed basic (alkaline) solution from the cathode stream (pH 10-11.5, ORP -800 to -900 mV, ACC 0 mg/L, and high dissolved hydrogen) which has a strong reducing potential is obtained (Kitano et al., 2003; Hsu et al., 2004; Al-Haq et al., 2005). The BEW is classified as a cleaning compound and used in clean-in-place (CIP) applications or as the primary cleaner on food processing equipment (Al-Haq et al., 2005; Rivera, 2005; Pangloli et al., 2009). The different researches which were published between 2003 and 2015, regarding physicochemical properties of neutral, near-neutral, and slightly electrolyzed waters applications in washing step of fruits and vegetables is summarized in Table 1.

DECONTAMINATION EFFECT OF ELECTROLYZED OXIDIZING WATER

The EOW term has also been classified as antimicrobial functional water and some scientists have used the different terminology for different types of electrolyzed water. Until today, different kinds of electrolyzed water (acidic, strong acidic, basic - alkaline, neutral, near neutral, or slightly acidic -low concentration) have been introduced as sanitizers and antimicrobial agents. In some studies of vegetable sanitization techniques which have been applied in fresh-cut produce, different types of electrolyzed water were used (Gómez-López et al., 2007; Afari et al., 2016; Zhang et al., 2016). EOW is produced with the electrolysis of diluted sodium, magnesium or potassium chloride solutions in a generator chamber (Figure 1), but sodium chloride is generally used for food applications (Koseki & Itoh, 2000; Al-Haq & Gómez-López, 2012; Gómez-López, 2012). EOW has an antimicrobial activity against pathogens and spoilage microorganisms (Vandekinderen et al., 2009; Rahman et al., 2010a; Graça et al., 2011) and has the same or more disinfectant ability as that of chlorine.

Although the antimicrobial mechanism of EOW has not been completely understood, there are some important factors such as electrochemically produced active chlorine derivatives (dissolved Cl₂ gas, HOCl, and OCl⁻), reactive compounds and short-lived radicals (ozone, O[•], Cl[•] and OH[•]) and ORP and low pH (Al-Haq et al., 2005; Koide et al., 2009; Zhang et al., 2011).

Table 1 Physicochemical properties of neutral, near-neutral, and slightly electrolyzed water which was used in published researches

Name or solution type	Abbreviation	pH	ORP (mV)	Available chlorine content (mg/L)	References
Neutral electrolyzed water	NEW ¹	8.13	803	430	Deza et al. (2003)
	NEW ²	8.03	816	432	
	NEW ³	7.99	795	465	
	NEW ⁴	8.2	808	450	
Neutral electrolyzed water	NEW ¹	6.34	265.1	21	Cui et al. (2009)
	NEW ²	6.51	512.6	25	
Neutral electrolyzed water	NEW	8.74	721	281	Abadias et al. (2008)
Neutral electrolyzed water	NEW ¹	8.42	767	101	Graça et al. (2011)
	NEW ²	8.39	753	49	
Neutral electrolyzed water	NEW	7.0		410	Tomás-Callejas et al. (2011)
Neutral electrolyzed water	NEW	7.0	900	100	Martínez-Hernández et al. (2013)
Near-neutral electrolyzed water		6.5-6.7	800-900		Guentzel et al. (2008)
Slightly acidic electrolyzed water	SIAEW	5.0-6.5		10-30	Koide et al. (2009)

Slightly acidic electrolyzed water	SAEW ¹ SAEW ²	6.53 6.41	238.4 265.2	2 6	Cao et al. (2009)
Slightly acidic electrolyzed water	SAEW	5.8	948	21	Issa-Zacharia et al. (2010)
Slightly acidic electrolyzed water	LcEW	6.3	520	5	Rahman et al. (2010a)
Slightly acidic electrolyzed water	SAEW	5.98-6.48	817.5- 893.8	20-120	Zhang et al. (2011)
Slightly acidic electrolyzed water	SAEW ¹ SAEW ²	6.19 5.95	860.25 873.85	19.61 29.42	Liu et al. (2013)
Electrochemically activated water	ECAW	7.0	864	100	Yang et al. (2013)
Near-neutral electrolyzed water	NEO	6.5	847	155	Afari et al. (2016)
Electrolyzed water	EO	2.5-6.5	836-1067	15-50	Zhang et al. (2016)
Low concentration electrolyzed water	LcEW	6.2	500-520	5	Ding et al. (2011)
Neutral electrolyzed water	NEW	6.5	750-900	500	Rico et al. (2008)
Neutral electrolyzed oxidizing water	NEW	8.3	-	45	Gómez-López et al. (2007)
Low concentration electrolyzed water	LcEW	6.2	500-520	5	Rahman et al. (2010b)
Neutralized electrolyzed oxidizing water	NEW	5.07	862.2	90.5	Xiong et al. (2010)

^{1,2,3,4} Solution type used in the same research which has different physicochemical parameters

So, the antimicrobial mechanism of EOW has been attributed to these factors by most researchers. For instance, there are some studies that found a high correlation between the HOCl content of EOW and the antimicrobial effect of it (Len et al., 2000; Al-Haq & Gómez-López, 2012). On the other hand, Huang et al. (2008) concluded that the high ORP of EOW could cause the modification of metabolic fluxes and ATP production probably due to the change in the electron flow in the cells. In addition, Demirci & Bialka (2011) hypothesized that the low pH and high ORP sensitizes the membrane of the microorganisms allowing the active transfer of hypochlorous acid into the microbial cell. Similarly, these findings, Liao et al. (2007) reported that a theory for the inactivation of bacteria based on the high ORP of EOW causes damage to the cell membranes. Due to the several disadvantages of AEW, researchers are focused on other types of electrolyzed water such as NEW, NNEW, or SAEW. In last years, EOW studies have focused on evaluating and comparing the disinfection efficiency of SAEW and other EOWs and antimicrobial agents such as NaOCl solutions. Koide et al. (2009) investigated the disinfection efficacy of SAEW (20 mg/L free chlorine, pH 6.1) in comparison to the NaOCl (150 mg/L free chlorine, pH 9.6) solutions on the total aerobic bacteria in fresh cabbage and the reductions were found as 1.6 and 1.5 log CFU/g, respectively, which are significantly higher than washing with tap water. When compared with the antimicrobial effects of SAEW, NaOCl, and control tap water, the number of mold and yeasts decreased to 1.3, 1.0, and 0.4, log CFU/g, respectively. The difference was found as being significantly important between the antimicrobial effect of NaOCl and SAEW (P<0.05). Similarly, many studies have also shown that SAEW may have an equivalent or higher microbiocidal effect than the NaOCl solution (Cao et al., 2009; Kim et al., 2009; Koide et al., 2009). Issa-Zacharia et al. (2010) aimed to assess the sanitization potency of SAEW against pure cultures of *E. coli* and *S. aureus*, and then comparing the potency with the NaOCl solution. The surviving population of *E. coli* decreased with exposure time and only small differences were noted in the reduction rate of SAEW and NaOCl solution. Similarly, the surviving population of *S. aureus* decreased with the increase in exposure time and all tested solutions showed a similar sanitization effect to that of the observed when applied to *E. coli* (Issa-Zacharia et al., 2010).

Rahman et al. (2010b) evaluated the efficacy of SAEW on lettuce leaves, under various treatment conditions for the inactivation of *L.monocytogenes*, *E. coli* O157:H7, *S. aureus*, and *S. typhimurium* with different dipping times (1, 3, 5, 7, and 10 min) and pH values of (2.5, 4.0, 5.0, 6.0, and 9.0) at room temperature (23±2 °C), and compared them with AEW (data not shown in table). The pH, ORP, and ACC of the treatment solutions used in this study are presented in Table 2. For 1 min dipping, the reduction in bacterial count through treatment with SAEW was about 5.20, 5.15, 4.90, and 6.21 log units CFU/ml for *L. monocytogenes*, *S. typhimurium*, *E. coli* O157:H7, and *S. aureus*, respectively, while the bacterial counts for samples treated with AEW were reduced by

approximately 4.92, 4.82, 4.70, and 5.72 log units CFU/ml for same bacteria, respectively. The 1 min dipping time showed a higher log reduction in each bacterium than for those at 3, 5, 7, and 10 min. Thus, as dipping time increased, ACC reduced and the rate of log reduction decreased significantly (P<0.05). Results also indicated that SAEW containing 5 mg/L of activated chlorine was more effective than that of 50 mg/L AEW in reducing populations of bacterial strains, regardless of dipping time (P<0.05) (Rahman et al., 2010b).

Table 2 Physicochemical properties of the treatment solutions (Rahman et al., 2010b)

Treatment solutions	pH value	ORP(mV)	Available chlorine concentration (ACC- mg/L)
DW*	6.63±0.05	410±12	0.50±0.08
AEW*	2.60±0.10	1100±20	50±2.2
SAEW*	6.30±0.20	500±20	5±0.1

* DW: Distilled water; AEW: Acidic electrolyzed water; SAEW: Slightly acidic electrolyzed water

DECONTAMINATION EFFECT OF EOW IN FRUIT AND VEGETABLE WASHING

The researches carried out previously, regarding electrolyzed water usage for decontamination in the washing step for fruits and vegetables are summarized in Table 3. Most of the researches were published in the years between 2006 and 2016. In these studies, the effects with different types of EOW such as acidic, neutral, near neutral or slightly acidic with different pH, ACC, ORP and treatment times were generally evaluated in the washing treatments of lettuce, cabbage, spinach, leek, Chinese celery, bean, mushroom, broccoli, apples, and radish. Consequently, it can be said that the washing applications with electrolyzed water at different ACC, ORP, treatment times, and pH values are not interpreted in the same baseline for all experimental conditions because of the different foods, test microorganisms, applications, parameters of each experiment. Decontamination effect of different types of electrolyzed water is primarily depend on these different parameters which has been used in treatments.

There are some studies designed to investigate and compare the impact of electrolyzed water with other decontamination agents such as organic acids, sodium hypochlorite, gaseous chlorine dioxide, and peroxyacetic acid on the microbial inactivation of some fruits and vegetables (Rahman et al., 2010a; Ding et al., 2011; Martínez-Hernández et al., 2013)

Table 3 Different electrolyzed water applications and microbial reductions in the washing decontamination process of fresh fruits and vegetables (summarized from published researches)

Food	Microorganism / Group	Reduction (log CFU/g)	Type of electrolyzed water/Treatment	pH	ACC (mg/L)	ORP (+mV)	References
Lettuce	Psychrotrophs	1.9	Neutral/1 min	-----	3.6	----	Ongeng et al. (2006)
	Lactic acid bacteria	1.2					
	Enterobacteriaceae	1.3					
	Mould and Yeast	0.11					
	Psychrotrophs	3.30	Neutral/5 min				
	Lactic acid bacteria	2.60					
	Enterobacteriaceae	1.90					
	Mould and Yeast	0.30					
TVC*	0.21	Neutral/5min-centrifuge	8.3	45	-----		

Cabbage	Lactic acid bacteria Psychrotrophs Yeasts	0.53 0.40 0.28					Gómez-López et al. (2007)
Iceberg lettuce	<i>E. coli</i> <i>Salmonella</i> <i>L. innocua</i>	1.60 1.50 1.30	Neutral/1-3 min -1 min rinsing with deionized water	8.6	52	722	Abadias et al. (2008)
	<i>E. coli</i> <i>Salmonella</i> <i>L. innocua</i>	1.80 1.30 1.40		8.55	89	733	
Spinach leaves	<i>E. coli</i>	0.44 1.25 2.62	Neutral/10 min -rinsing with sterile water	6.5- 6.7	4 20 50	800-900	Guentzel et al. (2008)
	<i>S. aureus</i>	0.52 2.04 3.47		6.5- 6.7	4 20 50	800-900	
	<i>S. Typhimurium</i>	3.41 2.14 2.28		6.5- 6.7	4 20 50	800-900	
	<i>L. monocytogenes</i>	3.77 2.94 ≥4.97		6.5- 6.7	4 20 50	800-900	
	<i>E. faecalis</i>	2.9 2.86 ≥4.33		6.5- 6.7	4 20 50	800-900	
Lettuce	<i>E. coli</i>	0.06 0.14 0.15	Neutral/10 min -rinsing with sterile water	6.5- 6.7	4 20 50	800-900	Rico et al. (2008)
	<i>S. aureus</i>	0.03 1.43 2.79		6.5- 6.7	4 20 50	800-900	
	<i>S. Typhimurium</i>	0.83 1.41 2.90		6.5- 6.7	4 20 50	800-900	
	<i>L. monocytogenes</i>	0.51 2.99 2.53		6.5- 6.7	4 20 50	800-900	
	<i>E. faecalis</i>	1.66 2.80 2.60		6.5- 6.7	4 20 50	800-900	
Iceberg lettuce	Mesophilic	2.20-2.40	Neutral/1 min -5 min drying with salad spinner	6.5	12- 120	750-900	Rico et al. (2008)
Leek	TVC	0.38 0.43	Neutral/1-5 min	7.5 7.8	4.5 30	-----	Vandekinderen et al. (2009)
Cabbage	TVC	1.50	Slightly acidic/10 min-rinsing with tap water /2 min	6.1	20	-----	Koide et al. (2009)
Spinach	TVC Mould and Yeast <i>E. coli</i>	1.93 1.64 2.40	Slightly acidic/ 3 min	6.2	5	500-520	Rahman et al. (2010a)
Chinese celery, Lettuce, sprout	TVC	2.70 2.54 2.45	Slightly acidic/ 5 min	5.8	21	948	Issa-Zacharia et al. (2011)
Mung bean	<i>E. coli</i>	1.35 ^a 2.56 ^b 3.41 ^c 4.37 ^d	Slightly acidic/ 5 min	6.13- 6.38	20 40 60 80	817-893	Zhang et al. (2011)
	<i>S. enteritidis</i>	1.31 ^a 2.13 ^b 3.28 ^c 4.23 ^d	Slightly acidic/ 5 min	5.98- 6.27	20 40 60 80	837-889	
Minzuna baby leaves	Mesophilic	0.02 0.38	Neutral/ 2min -rinsing with tap water/1 min		40 70		Tomás-Callejas et al. (2011)
	Enterobacteria	1.00 1.30	Acidic/2 min - rinsing with tap water/1 min		40 70		
		1.20	Neutral/2 min - rinsing with tap water/1 min		100		
	Psychrophilic	1.89	Acidic/2 min- rinsing with tap water/1 min		40		
		1.77	Neutral/2 min- rinsing with tap water/1 min		40		
	Mould and Yeast	1 1	Acidic/2 min- rinsing with tap water/1 min		40 70		

		1 1 1 1	Neutral/2 min- rinsing with tap water/1 min		100 40 70 100		
Apple	<i>E. coli</i>	1.90 ^a 1.40 ^b 1.20 ^{bc} 0.99 ^c	Acidic/5 min Acidic/5 min Neutral/5 min Neutral/5 min (3 min rinsing with distilled water)	2.93 3.08 8.42 8.39	98 53 101 49	1128 1111 767 753	Graça et al. (2011)
Mushroom	TVC <i>E. coli</i> <i>L. monocytogenes</i> <i>S. Typhimurium</i> <i>B. cereus</i>	1.35 1.85 2.16 2.08 2.02	Slightly acidic/3 min	6.2	5	500-520	Ding et al. (2011)
	TVC <i>E. coli</i> <i>L. monocytogenes</i> <i>S. Typhimurium</i> <i>B. cereus</i>	1.40 1.78 2.08 2.04 1.95	Strong acidic/3 min	2.5	50	1100-1120	
Broccoli	Mesophilic Psychrophilic Mould and Yeast	1.90 1 ≥3.0	Neutral/2 min- rinsing with tap water/1 min	7	100	900	Martínez-Hernández et al. (2013)
Lettuce	<i>S. Typhimurium</i> DT 104 <i>E. coli</i> O157:H7	5.00 3.90	Near neutral/15 min	6.5	155	847	Afari et al. (2016)
Radish	TVC Mould and Yeast	2.50 ^a 2.20 ^a 2.20 ^b 2.20 ^b	Electrolyzed water/12 h soaking	2.5 6.5 2.5 6.5	25	—	Zhang et al. (2016)

*Total Viable Count

The results of Vandekinderen et al. (2009) demonstrate that the reduction of the viable cells washed with different concentrations of some antimicrobials except peroxyacetic acid (250 mg/L) and chlorine dioxide gas (1.59 mg/L) was not significantly different. In addition, a reduction of approx. a 1.5 logarithmic unit was obtained for washing with a peroxyacetic acid (250 mg/L) and chlorine dioxide gas (1.59 mg/L) treatment, whereas, an 0.43 log unit was obtained after washing a fresh-cut leek with neutral electrolyzed water (30 mg/L ACC) for 1-5 min (Table 4).

Rahman et al. (2010a) investigated how to inactivate some microorganisms on spinach with washing with a slightly acidic (pH 6.2) and strong acidic (pH 2.54) EW and then compared it with other sanitizers such as aqueous ozone, citric acid, and sodium hypochlorite for 3 min at 23 °C (Table 5).

For all microorganisms, the similar pattern of microbial reduction was observed after slightly and strong acidic EW washing, no significant differences were determined among different washing treatments (P>0.05). The results of this research demonstrate that the slightly acidic EW washing (5mg/L ACC) can effectively reduce the number of *E. coli* and *L. monocytogenes* counts (2.4 and 2.8 log CFU/g reduction) on spinach when compared to the reductions of deionized water and sodium hypochlorite (100 mg/L ACC) washing with 0.80-0.95 and 2.0-2.2 log CFU/g reductions, respectively. As a result, slightly acidic

EW was proposed as a promising sanitizer for washing vegetables in that study (Rahman et al., 2010a).

Table 4 The efficiency of different disinfectants on the reduction of total viable count load of fresh cut leek (summarized from Vandekinderen et al., 2009)

Washing Treatment-Parameters	Reduction (log CFU/g)
Tap water (Control)	0.05±0.25
Neutral electrolyzed ACC water ACC	4.5 mg/L 30 mg/L 0.38±0.20 0.43±0.79
NaOCl ACC	20 mg/L 0.84±0.42
ACC	200 mg/L 1.00±0.64
Peroxyacetic acid	80 mg/L 250 mg/L 1.06±0.22 1.52±0.27
Chlorine dioxide gas	1.59 mg/L 1.48±0.48

The reduction values are given as means of log reduction ±SD

Table 5 The reduction values of different sanitizer washings for 3 min. on the total bacteria, mold and yeast, *E. coli*, and *L. monocytogenes* inoculated spinach samples (summarized from Rahman et al., 2010a)

Washing Treatment	Total bacteria (log CFU/g)	Mold and Yeast (log CFU/g)	<i>E. coli</i> (log CFU/g)	<i>L. monocytogenes</i> (log CFU/g)
Deionized water	0.52 ^a	0.31 ^a	0.80 ^a	0.95 ^a
Slightly acidic EW (SAEW) (5 mg/L ACC)	1.93 ^b	1.64 ^d	2.40 ^d	2.80 ^d
Strong acidic EW (StEW) (50 mg/L ACC)	1.94 ^b	1.57 ^d	2.30 ^d	2.70 ^d
Aqueous ozone (5.3 mg O ₃ /L water)	1.07 ^c	0.88 ^{bc}	1.22 ^{bc}	1.40 ^b
Citric acid (1 %)	1.39 ^{cd}	1.05 ^{cd}	1.50 ^c	1.70 ^b
NaOCl (100 mg/L ACC)	1.61 ^{db}	1.38 ^{cd}	2.01 ^d	2.20 ^c

^{a-d} Different subscripts show statistical significance (P<0.05) on the same column

In another study, Ding et al. (2011) investigated the effects of the same sanitizers which were used in Rahman's research (Rahman et al., 2010a) on the inactivation of same microbial groups except *S. typhimurium* and *B. cereus*. Samples treated with slightly acidic EW resulted in a greater log reduction than other sanitizers

for all microorganisms, there was no statistically significant difference between the slightly acidic and strong acidic EW results (Table 6).

The reduction of the initial microbial load of the fresh-cut broccoli after singular and combined decontamination treatments are given in

Table 6 The reduction values of different sanitizers on *E. coli* O157:H7, *L. monocytogenes*, *S. typhimurium*, and *B. cereus* inoculated oyster mushrooms during a 3 min. immersion treatment at room temperature (summarized from Ding et al., 2011)

Treatment	Total bacteria (log CFU/g)	Mold and Yeast (log CFU/g)	<i>E. coli</i> O157:H7 (log CFU/g)	<i>L. monocytogenes</i> (log CFU/g)	<i>S. typhimurium</i> (log CFU/g)	<i>B. cereus</i> (log CFU/g)
Deionized water	0.19 ^d	0.19 ^{cd}	0.39 ^e	0.62 ^d	0.66 ^c	0.57 ^c
Slightly acidic EW (pH:6.2 ORP 500-520 5 mg/L ACC)	1.35 ^a	1.08 ^a	1.85 ^a	2.16 ^a	2.08 ^a	2.02 ^a
Strong acidic EW (pH:2.5 ORP 1100-1120 50 mg/L ACC)	1.47 ^a	1.02 ^{ab}	1.78 ^a	2.08 ^a	2.04 ^{ab}	1.95 ^{ab}
Aqueous ozone (5 mg O ₃ /L water)	0.61 ^c	0.43 ^c	0.75 ^d	1.06 ^c	1.03 ^d	0.94 ^d
Citric acid (1 %)	0.9 ^{bc}	0.77 ^b	1.02 ^c	1.42 ^b	1.38 ^c	1.23 ^c
NaOCl (pH:9.8 100 mg/L ACC)	1.14 ^{ab}	0.88 ^{ab}	1.42 ^b	1.94 ^a	1.86 ^b	1.74 ^b

^{a-e} Different subscripts show statistical significance (P<0.05) on the same column

Martínez-Hernández et al. (2013) evaluated the alternative decontamination processes of fresh-cut broccoli and applied the following treatments (Tab. 7);

- Sodium hypochlorite (NaOCl) washing (100 mg/L free chlorine/2 min at 5 °C),
- Neutral electrolyzed water washing (NEW) (100 mg/L ACC, 900 ORP, 2 min at 5 °C),
- Super atmospheric oxygen packaging (HO) (90 kPa O₂ partial pressure),
- UV-C treatment (6 kJ UV-C/m²),

- Combinations of these treatments at same parameters (NEW+UV-C; UV-C+HO; NEW+HO and NEW+UV-C+HO).

Although it is generally accepted, as a rule, that the application of combined treatments could have a synergistic effect leading to a better microbial reduction. Martínez-Hernández et al. (2013) determined that the logarithmic reductions of mesophilic, psychrophilic, and mold and yeast counts is not significantly different from each other which are singular and combined washing treatments. The highest microbial reduction of combined washing treatments on mesophilic bacteria count was also obtained with NEW+HO (1.9 log CFU/g reduction) when compared with other treatments.

Table 7 The effect of singular and combined decontamination treatments applied to fresh-cut broccoli on the reduction (log CFU/g) of mesophilic, psychrophilic bacteria, and mold and yeast counts (summarized from Martínez-Hernández et al., 2013)

Washing treatment [†] (5°C- 2 min.)	Mesophilic bacteria (log CFU/g)	Psychrophilic bacteria (log CFU/g)	Mold and yeast counts (log CFU/g)
NaOCl (pH:6.5 100 mg/L ACC)	1.4 ^a	1.3 ^a	>3.1 ^a
UV-C Ultraviolet (6.0 kJ UV-C/m ²),	1.2 ^a	0.8 ^a	0.2 ^b
Neutral EW (NEW) (pH:7 100mg/L ACC)	0.5 ^a	1.3 ^a	>3.1 ^a
HO*	1.0 ^a	<10 ^a	>3.1 ^a
UV-C+HO	1.2 ^a	0.8 ^a	0.2 ^b
NEW+HO	1.9 ^b	1.3 ^a	>3.1 ^a
NEW+UV-C	1.3 ^a	1.2 ^a	>3.1 ^a
NEW+UV-C+HO	1.3 ^a	1.2 ^a	>3.1 ^a

* HO: super atmospheric O₂ packaging (active MAP with an initial 90 kPa O₂ partial pressure)

^{a-b} Different subscripts show statistical significance (P<0.05) on the same column

Table 8 The effect of electrolyzed water at different pH levels and the ACC at 22 °C for a 12 hours soaking treatment of radish on the total aerobic bacteria, and mold and yeast counts (summarized from Zhang et al., 2016)

Electrolyzed oxidizing water (EOW)	Total aerobic bacteria (log CFU/g)	Mold and yeast (log CFU/g)
Experiment 1		
25 mg/L ACC pH 2.5	2.46 ^a	2.21 ^b
25 mg/L ACC pH 6.5	2.17 ^a	2.27 ^b
Experiment 2		
15 mg/L ACC pH: 6.5	2.06 ^a	2.11 ^a
33 mg/L ACC pH: 6.5	2.40 ^b	2.58 ^b
40 mg/L ACC pH: 6.5	3.09 ^c	3.29 ^c

^{a-c} Different subscripts show statistical significance (P<0.05) on the same column

Zhang et al. (2016) investigated the efficacy of EOW in reducing the total aerobic count and yeast and mold counts on radish samples during a 12 h soaking (Table 8). In the research, EOW with different ACC (15, 20, 28, 33, and 40 mg/L) and different pH levels (2.5, 3.5, 4.5, 5.5, and 6.5) were used to soak radish for 12 h and the reductions of microorganisms were determined. The results showed that the reduction of total aerobic bacteria and mold and yeast counts was not significantly different at different pH levels (2.5 - 6.5) of EOW at 25 mg/L ACC. Results also showed that the reduction of total aerobic bacteria and mold and yeast counts increased with the increasing ACC of EOW, while a significant difference was observed between 15, 33, and 40 mg/L ACC at a 6.5 pH level that was applied while soaking radish samples at 22°C.

CONCLUSION

The decontamination effect of electrolyzed water is dependent on the organic load, pH, and ORP concentration in free oxidants and treatment times. As many

researches have demonstrated that slightly acidic electrolyzed water with a near-neutral pH value exhibits an equivalent or higher bactericidal activity for some kinds of foods used in these researches compared to acidic electrolyzed water and sodium hypochlorite solution and could prevent cross-contamination of processing environments. It was also reported that slightly acidic electrolyzed water applied by itself showed 1.0-1.80 log unit microbial reduction on the number of TVC, mould and yeast counts, and some pathogens such as *E. coli*, *L. monocytogenes*, *S. typhimurium* and *B. cereus* in some vegetables. In light of this knowledge, further studies are required to determine the antimicrobial effect of slightly acidic electrolyzed water or other antimicrobial agents in order to compare the results for decontamination washing processes, and simulate typical commercial conditions which may then lead to the fruitful applications of it in the food industry. Application of electrolyzed water has been primarily focused on fruits and vegetables; its potential for surface decontamination of food products still requires further study and optimization. Especially, application parameters such as pH, ORP, temperature, treatment time, and active chlorine concentration, require optimization for washing minimally processed fruits and vegetables to increase the microbiocidal effect of electrolyzed water washing as a promising alternative technique.

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