

Electrolyzed Water Application in Fresh Produce Sanitation

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Abstract

Electrolyzed water has gained more interest recently in food sanitation and safety for plant pathogens control, seed treatment, post-harvest disease control, fungal control and foodborne pathogens reduction. Different electrolyzed water solutions including acidic, alkaline, and neutral electrolyzed water solutions were applied by many researchers. In addition, electrolyzed water solutions were applied in combined with other technologies such as mild heat, ultraviolet, ultrasonication, other antibacterial chemicals, etc. Electrolyzed water can be applied in different steps of crop production from seed treatment to post harvest disease control. This chapter focuses on electrolyzed water definition, production mechanisms, advantages and disadvantages, their impacts on plant pathogens, fungi, bacteria and foodborne pathogens, regulations, and future of electrolyzed water technology.

Keywords: Electrolyzed water, fresh produce, plant pathogens, foodborne pathogens, advantages and disadvantages

Introduction

According to the Centers for Disease Control and Prevention, 31 known foodborne pathogens and unspecified agents are responsible for infecting 48 million people, 128,000 hospitalizations and 3000 deaths per year only in the United States, which can cost more than 15.6 billion US dollars (CDC, 2016). Among 31 foodborne pathogens, five of them are contributing to acquired foodborne illnesses causing death which are, *Salmonella* (nontyphoidal), *Toxoplasma gondii*, *Listeria monocytogenes*, *Norovirus*, and *Campylobacter* spp. Nearly half of the foodborne illnesses are caused by fresh produce including fruits, vegetables, and nuts. Worldwide, 1.5 billion cases of illness, and 3 million deaths are occurred annually (Al-Haq et al., 2005).

In addition, demand for fresh produce and ready to eat products is increasing which can increase the risk of foodborne illnesses. Although hazard analysis critical control point (HACCP) has been implemented by food plants, still there is a high risk of foodborne illnesses outbreak. In near future Food Safety Modernization Act, and Produce Safety Alliance might support the industry to reduce the cross contamination and foodborne illnesses. However, still there is a strong demand for applying different methods of sanitation, and processing to inactivate and reduce the pathogens in

foods. The food industry has applied and studied different sanitation technologies through the food chain. There are numerous sanitizing chemicals in food industry, including chlorine bases chemicals, peroxide mixtures, quaternary ammonium compounds (QUATS), acid anionic, hydrogen peroxide, peracetic acid, and iodine (Taylor et al., 1999; Marriott, 2006; Al-Qadiri et al., 2016). There are several criteria for an applicable sanitizer including, its ability to significantly reduce the number of microorganisms, avoiding cross contamination, being compatible with processing practices and available technical capabilities, affordable, safe to use, with no or minimum impact on quality, and approved by regulatory agencies.

However, many of these technologies have disadvantages including, low efficacy, high cost, chemical residue, adverse impacts on nutritional value, quality and consumer acceptance. Electrolyzed water gained more attraction in recent years as one of the safe sanitizers in food industry.

History and Terminology

Electrolyzed water was first developed around 1900 in Russia for water regeneration, water decontamination and sanitizing the medical devices. However, it was used for the first time for food processing in soda industry in Japan in 1980 (Al-Haq et al., 2005; Hricova et al., 2008; Rahman et al., 2016). Electrolyzed reducing water (ERW) with pH of 8-10 has been developed for health improvement and studied in 1931 in Japan, and its first application in agriculture was initiated in 1954. It was applied for medical purposes as a health-beneficial water in 1960, and the Ministry of Health, Labour and Welfare of Japan confirmed that ERW was effective for chronic diarrhea, indigestion, abnormal gastrointestinal fermentation, antacid, and hyperacidity, in 1966 (Shirahata et al., 2012). With recent development in technology, industry have been attempted to improve the electrolyzed water technology, and it is becoming more popular, and gained more attraction as a promising non-thermal technology, particularly for food industry.

Zeng and Zhang (2010) classified the history of electrolyzed water development into five stages, which the last 2 stages were modified in this book chapter which is more relevant to food application as follow:

- Discovery of water electrolysis phenomena (1800s-1920s).

- Industrialized for hydrogen production for industrial application such as ammonia production and petroleum refining (1920s-1970s).
- Systematic innovations and improvement in the systems, proton exchange membrane to answer the military and space demands (1970s-present).
- Rapidly developing and improving the system for using electrolyzed waters in medical, and food industry (present).
- Rapidly increasing neutral electrolyzed water production units and companies due to the big demand in food industry (2010-present).

Different names have been used for electrolyzed water by researchers and industry including, acidic oxidizing water (AOW), acidic electrolyzed water (AEW, AcEW, AcE water), electrochemically oxidizing water, aqua oxidation water, chlor aqueous solution, electrolyzed oxidizing water (EO water), electronically generated chlorine water, electronically prepared chlorine water (EPCW), electrolyzed strong acid aqueous solution (ESAAS), electrolyzed strong acid water (ESAW), functional water, activated water, redox water, sterilox water, strong ionized water, superoxide water, neutral electrolyzed water (NEW).

Production of Electrolyzed Water

Electrolyzed water, produced by electrolysis of a diluted sodium chloride solutions in an electrolysis chamber, divided by a diaphragm, which separates the anode and cathode. During electrolysis, sodium chloride dissolved in deionized water, which dissociated into Cl^- with negative charge, and Na^+ with positive charge. Meanwhile, water molecules are electrolyzed and formed hydroxide (OH^-), and hydrogen ions (H^+). Ions with negative charge (Cl^- and OH^-) move to the anode to give up the electrons and form oxygen gas (O_2), chlorine gas (Cl_2), hydrochloric acid (HCl), hypochlorite ion (OCl^-), and hypochlorous acid (HOCl). Positively charges ions (H^+ , Na^+) move to cathode to obtain electrons and become hydrogen gas (H_2), and sodium hydroxide (NaOH). At the end of the electrolysis process, two solutions are formed including acidic solution in anode, with a pH of 2 to 3, an oxidation-reduction potential (ORP) of more than 1000 mV, and an active chlorine content (ACC) of 10 to 90 ppm (depending on the salt concentration), and alkaline solution in cathode, with a pH of 10 to 13, and ORP of -800 to -900 mV (Al-Haq et al., 2005; Hricova et al., 2008) (Figure 1.6). Recently, industry and researchers have reported the generation of neutral electrolyzed water (NEW) with a pH of 7–8, and ORP of 750–1000 mV (Al-

Haq et al., 2005; Hricova et al., 2008), and slightly acidic electrolyzed water (SAEW) with a pH of 5–6.5 and ORP of approximately 850 mV (Nan et al., 2010). NEW is produced by mixing the anodic solution with OH^- ions or by electrolysis of NaCl in a single-cell unit (Hricova et al., 2008; Rahman et al., 2016), while slightly acidic electrolyzed water (SAEW) is generated by electrolysis of HCl alone or in combination with NaCl in a single-cell unit (Forghani et al., 2015; Rahman et al., 2016).

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The Advantages and Disadvantages of Electrolyzed Water

Electrolyzed water has many advantages compared to other sanitizing technologies;

- 1- It can be generated on site and it is relatively inexpensive.
- 2- It provides electrolyzed water with consistent quality, which can also be stored and has one to two years shelf life.
- 3- It can produce by electrolysis of water with dilute salt solution such as NaCl, KCl, or MgCl_2 , which makes it safe for the environment (Koseki et al., 2002; Al-Haq et al., 2005).
- 4- Its application reduces the safety and cost issues with handling, storage and application of chlorine solution.
- 5- In case of NEW it is safer for operators and employees since it does not generate chlorine gas.
- 6- It is easy to modify the chlorine concentration to achieve desired concentrations based on the application.
- 7- It can convert to the regular water after application, without releasing harmful gases.
- 8- According to some researchers, electrolyzed water does not cause resistance in microorganisms. (Al-Haq et al., 2005).
- 9- It is more effective than chlorine (Koseki et al., 2001; Issa-Zacharia et al., 2011). Consequently, the formation of chloramines and trihalomethanes is less (Al-Haq et al., 2005).

- 10- It can also prevent enzymatic browning during storage of foods in modified atmospheric packaging (Koseki and Itoh, 2002; Gómez-López et al., 2007).
- 11- Electrolyzed water has less cytotoxicity and less impact on the quality attributes of food materials. In case of AEW, it is less corrosive and has less impact on quality compared to other acidic solutions.
- 12- NEW has many advantages due to its neutral pH and the available form of chlorine (Deza et al., 2003).
- 13- NEW gained USDA certificate for organic produce production.

Electrolyzed water, similar to other technologies has its own disadvantages including:

- 1- AEW is corrosive for some metals and synthetic resin.
- 2- Its efficacy reduces significantly when it becomes in contact with organic materials particularly proteins due to its reaction with protein (Iwasawa and Nakamura, 1999).
- 3- In case of AEW, the machine can generate chlorine gas which is not safe for the operator.
- 4- The instrument is expensive.
- 5- AEW contains free chlorine which is phytotoxic to plants and damage plants tissue which make its application in farms, impossible (Schubert et al., 1995).
- 6- Sub-lethal doses of AEW and NEW can trigger toxin production in mold such as deoxynivalenol (DON) in *Fusarium* (Audenaert et al., 2012).

In general, NEW has more benefits and less disadvantages compared to AEW which is due to its pH and available form of chlorine which can make it more effective, and less corrosive.

The Mechanisms of Antimicrobial Activity of Electrolyzed Water

Extensive research on electrolyzed water has been conducted by many researchers on cell suspension, contact surfaces, fresh produce, plants, live animals, poultry, seafood, meat and food plant (environmental sanitation). The results from these studies show that electrolyzed water is a promising technology for sanitation, disease control, and preventive control.

Antimicrobial mechanism of electrolyzed water has not been fully understood (Al-Haq et al., 2005; Hricova et al., 2008). The antimicrobial activity of electrolyzed water strongly depends on pH,

oxidation reduction potential (ORP) and the form and concentration of available chlorine (Al-Haq et al., 2005; Hricova et al., 2008; Rahman et al., 2016).

Electrolyzed water can be discussed as a hurdle technology since it has different parameters which are responsible for its antimicrobial properties. Figure 2.6 shows the biosphere of a bacterium in response to pH and ORP. Microorganisms have their biosphere (red) in which, can survive and grow, while in the blue area, electrolyzed water prevent their growth because of acidic condition, and high ORP. Generally, bacteria can grow in a pH rang of 4 to 9. Aerobic bacteria can grow at the ORP rang of +200 to +800 mV, and anaerobic bacteria grow between -700 to +200 mV (Hricova et al., 2008). In AEW, low pH reduces bacterial growth making the bacterial cell more sensitive to active chlorine by changing the cell membrane (Hricova et al., 2008). However, the presence of chlorine, the available form of chlorine, and ORP are the main contributors in bacterial inactivation (Al-Haq et al., 2005; Hricova et al., 2008).

High ORP in electrolyzed water causes modification of metabolic fluxes and ATP production, because of the change in electron flow in cell. Active chlorine can destroy the membrane of the microorganisms, decarboxylate the amino acids, inhibit of oxygen uptake and oxidative phosphorylation coupled with leakage of some macromolecules, inhibit the glucose oxidation by chlorine-oxidizing sulfhydryl groups, form of toxic N-chlorine derivatives of cytosine, disrupt protein synthesis, react with nucleic acids, purines, and pyrimidines, unbalance metabolism of key enzymes (Kiura et al., 2002; Koseki and Itoh, 2000; Mahmoud et al., 2004; Mahmoud, 2007; Hricova et al., 2008).

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Some researchers reported that AEW had similar antibacterial activities at different pH range between 2.6 to 7, against *L. monocytogenes*, and *E. coli* 0157:H7, when adequate chlorine (more than 2 ppm) was provided (Park et al., 2004). Some other researchers reported that the high ORP is the main reason in bacterial reduction (Liao et al., 2007; Huang et al., 2008). While, some other researchers reported bacterial inactivation in lower ORP. For example, Rahman et al. (2012), found 5 log reduction in bacteria using electrolyzed water with ORP between 500 to 700 mV. In addition,

Koseki et al. (2001) reported that the ORP is not the main factor for inactivation the bacteria, because ozone also has high ORP, while its antimicrobial properties is significantly less than electrolyzed water. It seems that the main reason for inactivating the bacteria in electrolyzed water is mainly because of the synergistic effect of different parameters, and also the available free chlorine (Huang et al., 2008).

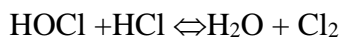
The antimicrobial efficacy of electrolyzed water strongly depends on different parameters including pH, available form of chlorine, ORP, current, water flowrate and salt concentration, storage condition, electrolyte and electrode materials, water temperature and hardness of water.

One of the advantages of using ORP for evaluating the properties of electrolyzed water, is its advantages to real-time monitoring the electrolyzed water antimicrobial potential. ORP could be measured by a probe in a real-time monitoring system, however, for chlorine determination, kits are required and it does not provide real-time information about the antimicrobial properties of the electrolyzed water.

Antimicrobial activity of electrolyzed water highly depends on pH and the fact that how pH can determine the available form of chlorine (Hricova et al., 2008; Rahman et al., 2016). Hypochlorous acid (HOCl) is the strongest form of chlorine, which shows 80 times greater sanitizing power than hypochlorite (ClO^-) when the pH is around 5 to 6.5 (Rahman et al., 2016). At lower pH, HOCl dissociated to Cl_2 gas, and at higher pH it forms ClO^- (Rahman et al., 2016) (Figure 3.6).

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The proportion of the HOCl and ClO^- in the water depends on the pH (Fig. 3). In alkaline conditions (pH. 7) ClO^- is the predominate chlorine type, while at pH values below 7, HOCl is the predominant part. At very low pH, formation of toxic Cl_2 gas occurs:



Active chlorine species including Cl_2 , ClO^- , and HOCl contribute in microbial inactivation. Fukuzaki (2006) explained the mode of action of chlorine. Technically, the main reason for

inactivation the bacteria, is the penetration properties of HOCl and ClO^- . Ionized ClO^- , is not able to penetrate the microbial cell membrane because of the existence of the hydrophobic lipid bilayer, some protective cell wall structures, and this fact that the cell of a pathogenic bacteria is negatively charged by nature. Negative charge of the hypochlorite ions (ClO^-) will be repulsed by the negative charge of the pathogenic bacteria cell wall, resulting in weak oxidizing action only outside of the cell. The neutral HOCl can penetrate the cell wall of the pathogenic microorganism very easily, thus making it a very effective disinfectant which can act on both outside and inside of the microorganism. HOCl can also penetrate slime layers, cell walls, and protective layers of microorganisms (Rahman et al., 2016). In addition, HOCl can kill bacteria by oxidizing sulfhydryl groups of certain enzymes, disruption of protein synthesis and oxidative decarboxylation of amino acids to nitrites and aldehydes.

The current, water flow rate and salt concentration also impacts the properties of electrolyzed water. Increasing water flow rate causes an increase in electric current due to more salt solution electrolysis (Hsu, 2003). Increasing bacterial reduction by increasing the water flow rate was reported for *E. coli* and *L. monocytogenes* (Rahman et al., 2012). The salt concentration has linear relationship with the chlorine concentration (Hsu 2005; Ovissipour et al., 2015; Rahman et al., 2016).

Application of all chlorine based sanitizers has one dramatic drawback, which is the evaporation of chlorine over time and HOCl breakdown, particularly in open conditions (Al-Haq et al., 2005; Hricova et al., 2008; Rahman et al., 2016). It has been also shown that even in sealed condition, due to the self-decomposition, the chlorine concentration reduced, however, it is significantly less than open condition (White et al., 1998). Rahman et al. (2012) showed that the antimicrobial activities of electrolyzed water retained up to 6 days, and 14 days, under open and closed storage conditions. Agitation can increase the chlorine loss during the storage by increasing the evaporation. For example, Len et al. (2002) reported that the electrolyzed water lost all chlorine after 30 h of agitation. It was shown that electrolyzed water stored at refrigerated temperature was more stable than one stored at 25 °C (Fabrizio and Cutter, 2003). The form of electrolyzed water has significant impact on the shelf life. Generally, the shelf life of the NEW is significantly more than AEW (Nagamatsu et al., 2002; Cui et al., 2009).

The Effect of Electrolyzed Water on Pre- and Post-Harvest Microorganisms Inactivation

Controlling the plant disease in pre- and postharvest, and in greenhouses, can reduce waste, increase the profit and provide secure food for human. Moreover, there is a big concern about using pesticides due to their impact on environment, workers' safety, fungicide resistances, and public health (Al-Haq et al., 2005). Hence, there is a big demand for developing green, and environmental friendly solutions as fungicide. Electrolyzed water has been applied by many researchers for plant disease control in pre-and post-harvest stages. For example, Grech and Rijkenberg (1992) injected AEW into a citrus micro-irrigation system to control water-borne pathogens, e.g. *Phytophthora* Spp., *Fusarium* Spp. algae, and skin forming bacteria. Their results showed that AEW was able to kill all the mentioned organisms. However, their results showed that nematodes were resistance to water chlorine. The effects of electrolyzed water on some organisms on plants are listed in Table 1.6.

Bonde et al. (1999) studied the effect of AEW on germination of *Tilletia indica* spores in wheat, and observed that applying AEW for 20 min, eliminated fungi such as *Asperigillus*, *Cladosporium*, and *Penicillium* spp.

Buck et al. (2002) was able to inactivate 22 different fungal species with AEW, and reported that all thin-walled species were killed by AEW in 30 s, and thick-walled were reduced or killed in longer time (2 min).

Abbasi and Lazarovits (2006) reported that the tomato seeds were immersed in AEW for 1 and 3 min, significantly reduced the populations of *Xanthomonas campestris* pv. *Vesicatoria* from the surface of the seeds without affecting the seeds germination.

Bandte et al. (2016) studied the effect of electrolyzed water on tomato virus (*Pepino mosaic*) in irrigation water. They reported that exposing the fruits to electrolyzed water, can significantly reduce the virus and improve the quality and growth of the fruits.

Zarattini et al. (2015) studied the effect of NEW with the pH of 6.5 (HClO) and electrolyzed water with the pH of 9 (ClO⁻) on plants of Petite Havana SR-1 (*Nicotiana tabacum*), Fuji (*Malus domestica*) and parcel of *Malus domestica* Dallago gene expression and defense mechanisms. It has been reported that electrolyzed water can kill the plant pathogens, however it is not clear that this property is only due to the biocide activity or there is also a positive effect on the plant. They

found that electrolyzed water is able to induce resistance in plants, which its mechanism will be discussed in next topic in this chapter.

Table 1.6: List of organisms, plants and treatments applied for sanitation

Organism	Plant	Solution	Outcome	Reference
<i>Tilletia indica</i> Teliospore	Wheat	AEW 16 ppm ACC	Significantly increased the germination	Bond et al. (1999)
<i>E. coli</i>	Alfalfa, and Broccoli seeds germination	AEW, ACC: 66 ppm; pH: 2.7; ORP: 1161 mV	No significant reduction	Kim et al. (2006)
<i>Xanthomonas campestris</i> pv. <i>Vesicatoria</i> (bacterial spot pathogen, <i>Streptomyces scabies</i> (potato scab pathogen), <i>Fusarium oxysporum</i> f.sp. <i>lycopersici</i> (root rot pathogen)	Tomato	AEW, ACC: 35 ppm; pH: 2.6; ORP: 1025 mV	Significant reduction, fruits performance either enhanced or not affected	Abbasi and Lazarovits (2006)
Powdery Mildew	Gerbera Daisy	AEW, ACC: 49-54 ppm; pH: 2.6; ORP: 1053 mV	sprayed twice a week and when sprayed every other week, alternating with fungicides	Mueller et al. (2003)
- Relatively thin-walled species (e.g., <i>Botrytis</i> , <i>Monilinia</i>) - Thicker-walled, pigmented fungi (e.g., <i>Curvularia</i> , <i>Helminthosporium</i>)	Greenhouse water	AEW, ACC: 55 ppm; pH: 2.6; ORP: 1079 mV	- Totally killed - After 2 min, significantly reduced	Buck et al. (2002)
powdery mildew, downy mildew, greymould	commercial rose varieties (<i>Rosa</i> sp) (Orlando and Versilia varieties)	NEW, ACC: 50 and 75 ppm; pH: 5; ORP: 850 mV	Significant control, however, some curled leaflets appeared with this application.	Fernandez et al. (2011)
powdery mildew	Peach trees once a week	AEW, ACC: 10-20 ppm; pH: 2.5; ORP: 1050 mV	Significantly reduction	Schoerner and Yamaki (1999)
powdery mildew (<i>Sphaerotheca fuliginea</i> Pollacci)	leaves of cucumber (<i>Cucumis sativus</i> L. cv. Shapu 7)	AEW, ACC: 30, 40, 50 ppm; pH: 2.3; ORP: 1170 mV	Significantly reduction	Fujiwara et al. (1998a)
downy mildew (<i>Pseudoperonospora cubensis</i> Rostowzew)	cucumber (<i>Cucumis sativus</i> L, cv. Naoyoshi)	AEW, ACC: 32 ppm; pH: 2.8; ORP: 1120 mV	After 17 days, downy mildew was controlled perfectly	Fujiwara et al. (1998b)
Pepino mosaic virus	Tomato	EW, ACC: 0.2 and 0.5 ppm, for 60 and 30 min exposing	Significantly reduction, no side effect on color, and nutritional value	Bandte et al. (2016)
Defense gene expression	Plants of <i>Nicotiana tabacum</i> cv. Petite Havana SR-1 and <i>Malus domestica</i> cv. Fuji parcel of <i>Malus domestica</i> cv. Dallago	NEW, ACC: 250 ppm; pH: 6.5 and 9;	Increasing plant resistance, no side effect on plant performance	Zarattini et al. (2015)

Electrolyzed water also has been applied by many researchers as a post-harvest control system for increasing the shelf life, improving the quality, sanitation, controlling mold and foodborne pathogens, etc.

Acidic electrolyzed water was used in frozen state with different chlorine concentrations against *L. monocytogenes* and *E. coli* O157:H7 (Koseki et al., 2004a). The results showed that the iced electrolyzed water with 250 ppm chlorine had the highest bacterial reduction. However, this concentration of chlorine caused physiological disorders in lettuce.

Koseki et al. (2004b) reported that cucumbers and strawberry washed with alkaline electrolyzed water (pH 11.3) for 5 min and then immersed in AEW (pH 2.6) for 5 min showed a strong bacterial and fungal reduction, and it is more effective compared to ozone and sodium hypochlorite treatments.

Guentzel et al. (2010) studied the effect of electrolyzed water on grapes and peaches artificially inoculated with *Botrytis cinerea* and *Monilinia fructicola*, respectively. The electrolyzed water with 25, 50, 75, and 100 ppm free chlorine and exposure time of 10 min were able to induce 6 log spores per ml reduction.

Application of electrolyzed water for apples, did not prevent lesion formation on fruit previously inoculated with *Penicillium expansum*, but cross-contamination of wounded apples from decayed fruit or by direct addition of spores to a simulated dump tank was significantly reduced (Okull and Laborde, 2004).

Whangchai et al. (2010) studied the effect of electrolyzed water on the reduction of *Penicillium digitatum* growth on tangerine. They reported electrolyzed water can totally deactivate the spores within one min.

In commercial trials conducted in Sicily (Italy) a 93% reduction of *Penicillium* spp. population in citrus wash water was observed 1 h after treating with electrolyzed water when water was supplemented with 1.25% of sodium bicarbonate (SBC); whereas, in the electrolyzed tap water without any salt, similar results were observed after 7 h. In addition, no rot development was observed in fruit exposed to electrolyzed SBC solution, whereas in the absence of salt, the rotten rate was 70% (Fallanaj et al., 2013).

Jemni et al. (2014) studied the effect of UV-C, ozone, and electrolyzed water on quality of palm. Their results showed that all treatments were able to decrease the microbial load significantly.

Lee et al. (2014) used NEW for removing indigenous flora on cabbage and carrot both in laboratory and processing line. In laboratory scale, they studied the effect of different hypochlorous acid concentrations (100, 150, and 200 ppm), different ratio of sample weight to NEW volume (1:5, 1:10, and 1:20), and different exposing times (5, 10, 20, and 30 min), using 2 kg of shredded cabbages and carrots. In processing line study, the feasibility of the NEW treatment was studied on an actual processing line (20 kg), including cutting, three washing steps (two air bubble washes for 5 min each and 150 ppm NEW for 5 min at ratio of 1:10), rinsing (5 min), and dehydration (5 min). Overall, more bacterial reduction was observed when HOCl concentration, and treatment time, were increased. The results showed 3.3–3.5 log CFU/g reductions at maximum conditions (NEW 200 ppm, 1:20, 30 min) in the coliform counts, however, some changes in color of both carrot and cabbage were reported.

Ding et al. (2015) studied the effect of slightly acidic electrolyzed water (SAEW) and ultrasound on microbial loads of fresh cherry tomatoes and strawberries. They found that, ultrasound can improve the antibacterial properties of SAEW significantly.

Vasquez-Lopez et al. (2016) investigated the NEW impacts on tomato rot (*Fusarium oxysporum*, *Galactomyces geotrichum*, and *Alternaria* sp.). The NEW chlorine concentrations were 10, 30, and 60 ppm, and fruits were exposed to the solutions for 3, 5, and 10 min. NEW with 60 ppm chlorine is effective enough to control the fungal rot in tomatoes.

The Effect of Electrolyzed Water on Bacterial Inactivation on Fresh Produce

The market for fresh produce is growing in the food industry, which is due to the healthy diet development in restaurants, changing life style and increased awareness of the importance of healthy diet. Particularly for the fresh-cut produce, fruits, ready salads, etc. there is a big demand in the market, however, fresh-cut produce market is limited because of their short shelf life and quality decline in post-processing, which is due to the biochemical changes associated with wounding compared to intact vegetables. On top of the post-harvest spoilage, frequency of foodborne illness outbreaks associated with fresh produce has increased due to more demands for

fresh produce (Rahman et al., 2011). This might be due to the processing steps such as peeling and cutting, which can increase the risk of cross contamination. In addition, initial microbial load, harvest methods, harvest region, water source can influence the final product microbial load. Water is used in postharvest processing of fresh produce to remove dirt and soil, cool, hydrate, and transport product, and if the water becomes contaminated with microbial pathogens, cross contamination occurs among the products and equipment surfaces. Sanitizing during the washing steps can help to control microbial hazards. Chlorine is one of the most widely used antimicrobial in minimally processed fresh produce processing. However, reaction of chlorine with some compounds in food can lead to the formation of carcinogenic chlorinated compounds, and therefore there is a need for finding an alternative. Different sanitation methods have been applied for fresh produce, including rinsing produce in lemon juice and lemon juice vinegar (Sengun and Karapinar, 2004, 2005), anolyte water and chlorinated water (Workneh et al., 2003). Chlorine dioxide, ozone, and thyme essential oil have also been used to sanitize the produce (Singh et al., 2002). A 3-log reduction of microbial load was observed in response to heat treatment (Alegria et al., 2009, 2010), acidified sodium chlorite (Ruiz-Cruz et al., 2007), peroxyacetic acid (Vandekinderen et al., 2009), and irradiation (Chaudry et al., 2004) treatment. Warm water (Klaiber et al., 2004) and electrolyzed water (Izumi, 1999) have also been found to decrease the populations of bacteria.

Electrolyzed water has been applied for killing foodborne pathogens, although it has some limitations like other disinfectants for the inactivation of microorganisms in whole and minimally processed produce (Gómez-López et al., 2008a). Venczel et al. (1997) reported the inactivation of *C. perfringens* spores by NEW. Venkitanarayanan et al. (1999) reported the inactivation of cultures of *E. coli* O157:H7, *Salmonella enteritis*, and *L. monocytogenes* by approximately 7 log CFU/ml using AEW. Subsequent studies have also proved the efficacy of EO water to inactivate human pathogens both in vitro (Nakajima et al., 2004; Ovissipour et al., 2015) and inoculated onto vegetable surfaces (Deza et al., 2003; Sharma and Demirci, 2003; Abadias et al., 2008), AEW and NEW for contact surfaces (Al-Qadiri et al., 2016), food processing (Shiroodi et al., 2016; Ovissipour et al., 2017). The effect of AEW with 30 ppm chlorine and water with 200 ppm chlorine was studied on *E. coli* O157:H7, *S. enteritis*, and *L. monocytogenes* on the surfaces of tomatoes. The results showed that, water with 200 ppm chlorine and AEW reduced the number of pathogens by 4.69–4.87 log CFU and 7.46–7.85 log CFU per tomato, respectively (Bari et al., 2003).

Guentzel et al. (2008) reported 4.0–5.0 log reductions of *E. coli*, *Salmonella typhimurium*, *S. aureus*, *L. monocytogenes*, and *Enterococcus faecalis* on spinach after dipping for 10 min in NEW at 100 and 120 ppm total residual chlorine. However, they reported limit of efficacy for lettuce surface with 0.25 log reduction for *E. coli* and 2.43–3.81 logs for the rest. For minimally processed produce, AEW and NEW were able to decrease the number of *E. coli* O157:H7, *L. innocua* and *Salmonella choleraesuis* inoculated individually and in a mixture on apples by 1.2–2.4 logs, a limited decontamination level but equal or more effective than that achieved with sodium hypochlorite (Graca et al., 2011). On strawberries, EO water was as effective as chlorinated water for inactivation of *E. coli* O157:H7 cells (Hung et al., 2010).

Deza et al. (2003) reported the effectiveness of NEW treatment on tomatoes against *E. coli*, *S. typhimurium*, *L. monocytogenes*, and *Salmonella enteritidis* with more than 5 log CFU/cm² reduction. In strawberries, 0.96 and 0.93 log reductions were achieved for yeasts and molds and total aerobic bacteria, respectively, upon treating with SAEW containing 34 ppm active chlorine at pH 6.49 (Ding et al., 2015). These results are similar to those reported by Hao et al. (2011), which the treatment of fresh-cut cilantro in SAEW for 5 min resulted in 1.56 and 1.64 log CFU/g reductions in total aerobic bacteria and yeasts and molds, respectively. They reported that, SAEW is a promising food sanitizer that may be considered as an alternative to NaOCl solution and would reduce the amount of active chlorine used in fresh produce.

Apples and their products which are contaminated with the common storage rot fungus *Penicillium expansum*, contain patulin which is a mycotoxin. Using 100% or 50% EO water containing 60 ppm free chlorine could reduce *P. expansum* viable spore populations by greater than 4 and 2 log in aqueous suspension and wounded apples, respectively (Okull and Laborde, 2004). EO water was able to control brown rot in wound-inoculated fruits, but reduced disease incidence.

Koseki et al. (2004b) reported that EO water did not reduce the bacteria in strawberry which might be due to the surface structure of the strawberry fruit. There are many achenes (seeds) that render its surface structure uneven and complex. These studies showed that the surface properties of fruits can strongly impact the efficacy of EO water.

Exposing time, chlorine concentration, pH, available form of chlorine, other technologies combined with electrolyzed water, agitation speed during the sanitation, chemical composition of produce can impact on the efficacy of the electrolyzed water against bacteria (Rahman et al., 2016).

Electrolyzed water can be used in combination with other technologies, and it has been shown that its efficacy can be improved in many cases.

Koseki et al. (2004c) used mild thermal processing (50°C) in combination with alkaline electrolyzed water for treating vegetables for 5 min and subsequent washing with chilled acidic electrolyzed water (4°C) for 1 or 5 min. They reported 3 to 4 log CFU/g reduction of *E. coli* O157:H7 and *Salmonella* on lettuce. Koide et al. (2011) used mild heated (45°C) and SAEW for sanitizing the sliced carrots and their results showed total aerobic bacteria, mold and yeast populations were significantly lower after mildly heated SAEW treatment.

Park et al. (2009) used 1% citric acid and alkaline electrolyzed water heated at 40°C, against *Bacillus cereus* both vegetative and spore form in brown rice and reported 4.21 and 3.57 log reduction, respectively.

Low concentration electrolyzed water heated at 40°C and ultrasound were used against *E. coli* O157:H7 in lettuce and 3.18 log reduction was reported, and the shelf life was improved.

Mansur and Oh (2015) studied the impact of temperature on the sanitizing efficacy of SAEW (ACC 5 ppm, pH 6.28, exposure time 3 min) on fresh-cut kale. The treatment resulted in >1.5 and 2 log CFU/g reduction in *L. monocytogenes* at 4 and 7°C, respectively.

Afari et al. (2015) studied NEW (155 ppm chlorine; pH: 7.52; ORP: 760) effect on *E. coli* O157:H7, and *S. typhimurium* CT 104 on fresh produce (Romaine and Iceberg lettuce, and tomato) using an automated washer at simulated food service conditions at different times (1 to 30 min) and different agitation speeds (40 and 65 rounds per min; rpm). They reported time and agitation speed significantly increased the bacterial log reduction.

Ding et al. (2015) used SAEW (33 ppm chlorine; pH: 6.48; ORP: 853), and ultrasound (40 kHz, 10 min) on cherry tomatoes and strawberries total aerobic bacterial count and different quality attributes. They reported that ultrasound can increase the efficacy of the SAWE.

Some researchers also found that the traditional sanitizers such as lactic acid (2%) showed higher antibacterial properties compared to AEW (Tirawat et al., 2016).

The Effect of Electrolyzed Water on Plant Physiology and Quality

Induce Resistance

Navarro-Rico et al. (2014) studied the effect of NEW and AEW on broccoli microbial load and total phenolic contents and reported that electrolyzed water could decrease the microbial load significantly, and increase the total phenolic contents up to 30%. Generally, electrolyzed water can be an abiotic stress that may induce a total phenolic content increase in the plant which can improve the resistance.

Zarattini et al. (2015) studied the effect of NEW at the pH of 6.5 (HOCl) and electrolyzed water at the pH of 9 (OCl⁻) on tobacco and apple gene expression and defense mechanisms. It has been reported that electrolyzed water can kill plant pathogens, however it is not clear that this property is only due to the biocide activity or there is also a positive effect on the plant. They found that electrolyzed water is able to induce resistance in plants, which its mechanism will be discussed in next topic in this chapter. They determined the genes which are responsible for the defense against fungi, and reported that member of the PR genes have the key role. They exposed the plants to electrolyzed waters at different time intervals and measured the level of gene expression. They reported that gene expression was elevated only for 6 h after first treatment, 48 h after second treatment (PR changed 40 times) (14 days after first treatment), and 96 h after third treatment (PR changed 100 times) (35 days after first treatment). It has been reported that for tobacco plant, the gene expression depended on the concentration of chlorine in electrolyzed water. For example, the highest gene expression was observed in plants treated by 250 ppm, and weak gene expression was observed in plants treated by 125 and 500 ppm, suggesting that 250 ppm is the optimal concentration for tobacco plants. In addition, they found that the available form of chlorine which depends on the pH of the solution, has significant impact on the gene expression. At neutral pH, chlorine is available as hypochlorous acid (HOCl), while at alkaline pH it is mainly hypochlorite (OCl⁻). After treating the tobacco with electrolyzed waters with different pHs including 6.5 (HOCl) and 9 (OCl⁻), they reported that alkaline electrolyzed water triggers an overexpression that is limited to some of the PR genes such as PR1a, and PR2, while other genes are not upregulated. After the second treatment (14 days after the first one), PR1a, and PR2 showed 100 times and 10 times increase, respectively after treating with alkaline solution, while in the case of neutral pH (HOCl), 1000 times and 100 times increase were reported. These results indicate that hypochlorous acid is essential to achieve a strong and long-lasting activation of plant defense. The mechanism is not understood very well, however, researchers claimed that this might be due to the increase in salicylic acid production which is an important hormone and acts as the endogenous defenses

activator. The salicylic acid concentration increased ten times in samples treated with NEW compared to the control group, which might improve the defense system at least partially.

In another study, same researchers applied electrolyzed water in chamber on one-year old apple tree and in orchard on 20-year-old apple tree. They found the same results as tobacco and reported that electrolyzed water was able to trigger a defensive response in apple trees in first exposure. Interestingly, gene expression was higher in trees in orchard compared to in chamber ones.

Fallanaj et al. (2016) studied the effect of electrolyzed water, and electrolyzed water with NaHCO_3 on green mold inactivation, and inducing resistance in citrus fruits against green mold. Activity and gene expression of phenylalanine ammonia-lyase, peroxidase, chitinase, and β -1,3-glucanase, in fruit tissue were evaluated and results showed an increase in the activity of all tested enzymes in treated tissue at 12–24 h post treatment, as compared to the control fruit. Peroxidase and phenylalanine ammonia-lyase activity were strongly activated in electrolyzed water with NaHCO_3 in treated tissue at 12 and 24 h post treatment. Both enzymes are considered important in host resistance mechanisms, since peroxidase is involved in lignin formation and phenylalanine ammonia-lyase is the first enzyme involved in the phenylpropanoid pathway, which helping fruit tissues to better respond to pathogen attack by establishing biochemical defensive barriers. In addition, chitinase and β -1,3-glucanase activity were increased by electrolyzed water with NaHCO_3 as compared to the other treatments. Chitinase and β -1,3-glucanase are able to hydrolyze fungal cell components (chitin and glucans), and, in combination, they have been shown to inhibit the growth of several pathogenic fungi (Schlumbaum et al., 1986; Sela-Buurlage et al., 1993). Gene expression was used to confirm the biochemical results. The relative expression of peroxidase and phenylalanine ammonia-lyase genes was higher in electrolyzed salt-treated tissues as compared to the other treatments. In particular, on tissue treated with electrolyzed water with NaHCO_3 , the induction was maximum at 6 and 12 h post treatment for peroxidase and phenylalanine ammonia-lyase, respectively. The results from this study showed that, electrolyzed water with NaHCO_3 upregulated the same pattern of genes involved in the general response to stresses, such as salt stress or oxidative stress, so that induction caused by treatment might sum up to host natural defense mechanism.

Quality changes

One of the negative impacts of the electrolyzed water is their impact on quality, particularly during the post-harvest in fresh produce and fresh cut-produce.

Koseki and Itoh (2001) reported that cut vegetables subjected to immersion in AEW (42.3 mg/L available chlorine, pH at 2.5), NaOCl solution (150 mg/L available chlorine, pH at 9.3) or tap water (0.3 mg/L available chlorine, pH at 7.0) for 10 min showed 15 to 20% reductions in ascorbic acid content for cut cabbage, 10 to 15% reductions for cut lettuce and 30 to 35% reductions for cut cucumber.

Koide et al. (2011) used SAEW (23 ppm chlorine, pH: 5.5) alone and in combination with mild heat (45°C), on fresh-cut carrot. They did not observe any changes in color hue and chroma, hardness, ascorbic acid and β -carotene content.

Rahman et al. (2011) applied AEW in combination with 1% citric acid at 50°C for fresh-cut carrot. They reported that the combination of disinfectants improve the antimicrobial properties and increase the shelf life of the fresh produce.

Navarro-Rico et al. (2014) applied NEW, AEW, and NaClO (70 and 100 ppm chlorine) for fresh-cut broccoli and studied the shelf life and total phenolic compounds in 15 days of storage at 4°C. Total phenolic compounds in EW treated samples was 16 to 30% higher compared to NaClO treated samples. However, in contrast with these results, other researchers did not observe total phenolics changes after applying EW (Martínez-Hernández et al., 2013). They also studied the activity of different enzymes including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and glutathione reductase (GR). Among them, APX, and GPX activities after EW treatment did not show significant changes compared to NaClO. However, SOD, and CAT activities significantly decreased after applying EW around 13-37, and 40-46%, respectively, compared to NaClO. Technically, EW has shown strong and stable SOD- and CAT-like activities due to the high level of dissolved molecular hydrogen produced in EW during electrolysis of water. Hence, the SOD and CAT-like activities of EW could contribute to the scavenge ring of reactive oxygen species produced throughout the shelf life of the fresh-cut produce.

Jemni et al. (2014) studied the effect of UV-C in combination with ozone, and NEW (100 ppm chlorine; pH: 6.99; ORP: 870) on shelf life and quality of date palm during 30 days storage at 20°C. NEW and UV-C combination had the lowest weight loss after 30 days, compared to different doses of UV-C alone, and ozone and UV-C combination. UV-C combined with NEW and ozone showed highest total phenolic contents, and total sugar content.

Washing with AEW containing 16.8 ppm chlorine did not affect the color of cilantro leaves, however, the AEW treated samples stored at 0°C for 14 days showed less aroma than water-washed samples, which might be correlated to their high tissue electrolyte leakage (Wang et al., 2004).

The effect of different treatments including tap water, SAEW (20 ppm chlorine; pH: 5.85; ORP: 815), AEW (80 ppm chlorine; pH: 2.48; ORP: 1134), NaClO solution (103 ppm chlorine; pH: 10; ORP: 500), alone and in combination with heat (45°C) were applied for fresh-cut cilantro (Hao et al., 2015). They reported that SAEW showed the advantage in keeping the overall quality (electrolyte leakage, texture, and smell) compared to other treatments and it might be a better choice for fresh-cut cilantro compared to AEW. SAEW had higher pH and lower chlorine which might cause less cell damage in cilantro.

Ding et al. (2015) studied the effect of SAEW in combination with ultrasound on cherry tomatoes and strawberries and found that, except for firmness of cherry tomatoes which decreased, the other quality attributes including total soluble solids, total titratable acidity and vitamin C did not change.

Regulation

Legislation for process water sanitizer in the United States, may be regulated by FDA and/or USA Environmental Protection Agency (EPA) depending on the product which is washed and processing location. For fresh-cut produce, sanitizers are regulated by the FDA as a secondary direct food additive and for raw fresh produce that are washed in the fields, sanitizers are considered as “pesticides” that are regulated by the EPA.

Japanese Ministry of Health and Welfare approved AEW with 20 to 60 ppm chlorine, and SAEW with 10 to 30 ppm chlorine (Koide et al., 2009). FDA approved HOCl application 21 CFR 173.315 for chemicals used in washing or to assist in the peeling of fruits and vegetables. Hence, since the main compound in NEW is HOCl, it might be regulated by the same CFR. USDA authorized NEW application for organic products.

Future of Electrolyzed Water

Electrolyzed water applications in different sections have been already proved. It seems electrolyzed water has the potential for being used as one of the useful sanitizers in food, aquaculture, agriculture, medical and energy industry. Recently, many startup companies and industries started commercialization and marketing of different types of electrolyzed water all around the world.

There are many companies worldwide that have been established for producing pure electrolyzed water solutions with different chlorine concentrations for different applications. For example, **AquaOx LLC** in US is producing two different electrolyzed water with different hypochlorous acid concentrations which have been tested in food plants and for medical applications. Additionally, this company is using these solutions for treating plant diseases by spraying them on the trees. It seems in near future electrolyzed water could be sold in stores for using at home sanitation. The small electrolyzed water machines are also available which could be installed in restaurant and medicals offices for the purpose of sanitation and disinfection of contact surfaces and instruments.

Using electrolyzed water for treating plant disease or in aquaculture for sanitizing and treating pathogenic microorganism, can provide pesticide free and drug free fresh products for human consumption. Furthermore, electrolyzed water impacts on wound healing and its wound sanitation application have been approved, there are several companies producing diluted hypochlorous acid for wound treatment.

It is clear that electrolyzed water is one of the promising sanitizers for future, which can provide pesticide and drug free food products.

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