Application of electrolyzed water in the food industry

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Abstract

Electrolyzed oxidizing (EO) water has been regarded as a new sanitizer in recent years. Production of EO water needs only water and salt (sodium chloride). EO water have the following advantages over other traditional cleaning agents: effective disinfection, easy operation, relatively inexpensive, and environmentally friendly. The main advantage of EO water is its safety. EO water which is also a strong acid, is different to hydrochloric acid or sulfuric acid in that it is not corrosive to skin, mucous membrane, or organic material. Electrolyzed water has been tested and used as a disinfectant in the food industry and other applications. Combination of EO water and other measures are also possible. This review includes a brief overview of issues related to the electrolyzed water and its effective cleaning of food surfaces in food processing plants and the cleaning of animal products and fresh produce.
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Contents

1. Introduction

Food-borne illnesses are prevalent all over the world. The toll of that in terms of human life and suffering is enormous. Acute food-borne disease infections and intoxications are much more of a concern to governments and the food industry today than a few decades ago. From January 1988 through December 1997, a total of 5170 outbreaks of food-borne disease were reported to the Centers for Disease Control and Prevention. These outbreaks caused 163,000 persons to become ill (Bean, Goulding, Lao, & Angulo, 1996; Olsen, Mackinnon, Goulding, Bean, & Slutsker, 2000). Food-borne infections are estimated to cause 76 million illnesses, 300,000 hospitalizations and 5000 deaths annually in the USA (Mead et al., 1999). When excluding multi-ingredient foods, seafood ranked third on the list of products which caused food-borne disease between 1983 and 1992 in the USA (Lipp & Rose, 1997). Moreover, the top five food categories linked to food poisoning outbreaks in the USA from 1990 to 2003 were seafood, dairy products, eggs, beef, and poultry products which were responsible for 61% of all outbreaks according to the Center for Science in the Public Interest (CSPI)'s database (CSPI, 2006). Globally, the search for effective and safe protocols and agents for rendering food safety has been continued to engage the attention of researchers, food manufacturers and retailers as well as policy makers, in countries such as the USA, Japan, UK and Taiwan. In fact, recent outbreaks of food-borne illnesses in Taiwan, USA and Japan, have raised vast international concern.

The best way to reduce incidences of food-borne diseases is to secure safe food supply. Although Hazard Analysis Critical Control Point (HACCP) system has been implemented in many food processing establishments, most outbreaks of food-borne illnesses still occurred in foodservice sectors including institutions, fast food restaurants, and food stores, where food products had undergone various treatments and should have been rendered as safe (Chang, 2003). This situation indicates that hazards might still exist in the food supply systems. Today, food chains are becoming complicated in handling, processing, transportation, and storage ensuring a safe food supply becomes a challenge task.

Electrolyzed oxidizing (EO) water, also known as strongly acidic electrolyzed water (SAEW) or electrolyzed strong acid aqueous solution (ESAAS), is a novel antimicrobial agent which has been used in Japan for several years. It has been reported to possess antimicrobial activity against a variety of microorganisms (Fabrizio & Cutter, 2003; Horiba et al., 1999; Iwasawa & Nakamura, 1993; Kim, Hung, & Brachett, 2000a, 2000b; Kim, Hung, Brachett, & Frank, 2001; Kimura et al., 2006; Kiura et al., 2002; Park & Beuchat, 1999; Park, Hung, & Brackett, 2002a; Venkitanarayanan, Ezeike, Hung, & Doyle, 1999b; Vorobjeva, Vorobjeva, & Khodjaev, 2003). In recent years, EO water has gained interest as a disinfectant used in agriculture, dentistry, medicine and food industry. It has been shown as an effective antimicrobial agent for cutting boards (Venkitanarayanan, Ezeike, Hung, & Doyle, 1999a), poultry carcasses (Fabrizio, Sharma, Demirci, & Cutter, 2002; Park et al., 2002a), eggs (Russell, 2003), lettuce (Izumi, 1999; Koseki & Itoh, 2001; Koseki, Yoshida, Isobe, & Itoh, 2001; Koseki, Fujiwara, & Itoh, 2002; Koseki, Isobe, & Itoh, 2004a; Koseki, Yoshida, Kamitani, Isobe, & Itoh, 2004c; Park, Hung, Doyle, Ezeike, & Kim, 2001; Yang, Swem, & Li, 2003), alfalfa seeds, sprouts (Kim, Hung, Brackett, & Lin, 2003; Sharma & Demirci, 2003), pears (Al-Haq, Seo, Oshita, & Kawagoe, 2002), apples (Okull & Laborde, 2004), peaches (Al-Haq, Seo, Oshita, & Kawagoe, 2001), tomatoes (Bari, Sabina, Isobe, Uemura, & Isshiki, 2003; Deza, Araujo, & Garrido, 2003), strawberry (Koseki, Yoshida, Isobe, & Itoh, 2004b) and food processing equipments (Ayebah & Hung, 2005; Ayebah, Hung, & Frank, 2005; Kim et al., 2001; Park, Hung, & Kim, 2002b; Venkitanarayanan et al., 1999a; Walker, Demirci, Graves, Spencer, & Roberts, 2005a, 2005b). EO water also has the potential to be more effective and inexpensive than traditional cleaning agents. The greatest advantage of EO water for the inactivation of pathogenic microorganisms relies on its less adverse impact on the environment as well as users' health because of no hazard chemicals added in its production. Moreover, it has been clarified that EO water does no harm to the human body (Mori, Komatsu, & Hata, 1997). It is more effective, less dangerous and less expensive than most traditional preservation methods such as glutaraldehyde (Sakurai, Nakatsu, Sato, & Sato, 2003; Sakurai, Ogoshi, Kaku, & Kobayashi, 2002), sodium hypochlorite and acetic acid (Ayebah et al., 2005). Many aspects of EO water are elucidated in this review, including its chemical and physical properties, generation, antimicrobial properties and its applications in food industries, such as fresh vegetables, fruits, eggs, poultry and seafood.

2. Principles and characteristics of electrolyzed water

EO water was initially developed in Japan (Shimizu & Hurusawa, 1992). It has been reported to have strong bactericidal effects on most pathogenic bacteria that are important to food safety. EO water is produced by passing a diluted salt solution through an electrolytic cell, within which the anode and cathode are separated by a membrane. By subjecting the electrodes to direct current voltages, negatively charged ions such as chloride and
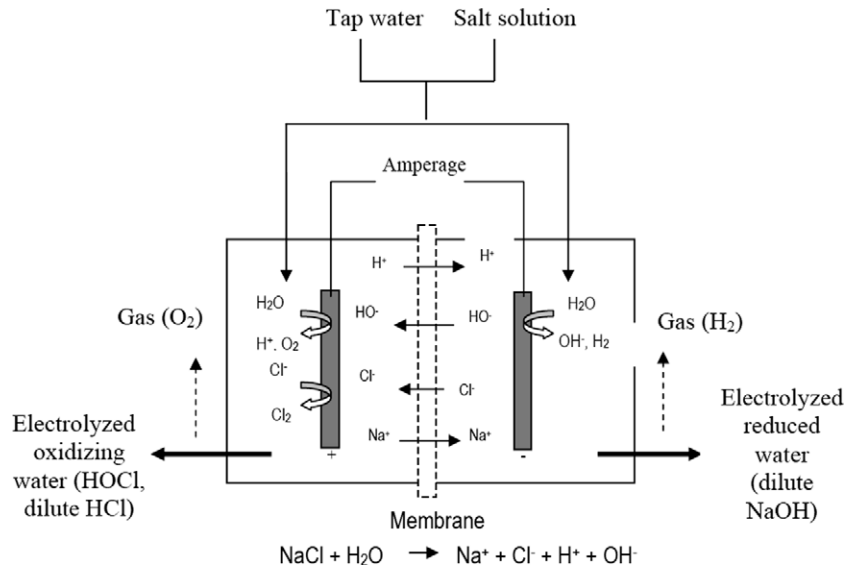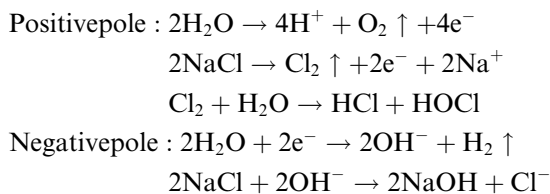
Fig. 1. Schematics of electrolyzed water generator and produced compounds.

hydroxide in the diluted salt solution move to the anode to give up electrons and become oxygen gas, chlorine gas, hypochlorite ion, hypochlorous acid and hydrochloric acid, while positively charged ions such as hydrogen and sodium move to the cathode to take up electrons and become hydrogen gas and sodium hydroxide (Hsu, 2005). Two types of water are produced simultaneously. EO water, with low pH (2.3–2.7), high oxidation–reduction potential (ORP, >1000 mV), high dissolved oxygen and contains free chlorine (concentration depends on the EO water machine setting), is produced from anode side. However, electrolyzed reduced (ER) water, with high pH (10.0–11.5), high dissolved hydrogen, and low ORP (−800 to −900 mV), is produced from the cathode side. ER water with strong reducing potential can be used to remove dirt and grease from items such as cutting boards and other kitchen utensils (Hsu, 2005).

The principle of producing electrolyzed water is shown in the Fig. 1 with the following:

$$Positive pole : 2H_2O \rightarrow 4H^+ + O_2 \uparrow +4e^-$$
$$2NaCl \rightarrow Cl_2 \uparrow +2e^- + 2Na^+$$
$$Cl_2 + H_2O \rightarrow HCl + HOCl$$
$$Negative pole : 2H_2O + 2e^- \rightarrow 2OH^- + H_2 \uparrow$$
$$2NaCl + 2OH^- \rightarrow 2NaOH + Cl^-$$

3. Systems for generation of electrolyzed water

Commercial EO water generators can be divided into three major types based on their automatic control systems. The first type of EO water generators, made by the ARV® and the Amano® companies, allows the users to select brine flow rate while the machines adjust voltages and/or amperages automatically. The second type of EO

water generators, made by the Hoshizaki® Company, allows the users to select amperages and/or voltages, while the machines change brine flow rate accordingly. The third type of EO water generators, made by the Toyo® and the Nippon Intek® companies, allows the users to select a pre-set chlorine concentration level of EO water from a display panel and the machines change brine flow rate and amperages and/or voltages automatically (Hsu, 2003).

Hsu (2003) investigated relationship among water flow rate, water temperature and salt concentration on electrolysis efficiency, and separation efficiency of an EO water generator. He made following conclusions: (1) electric potential (7.9–15.7 V) and power consumption (16–120 W) of electrolysis cell were not affected by water flow rate, water temperature or salt concentration in the feed solution; (2) electric current changed with water temperature and water flow rate; and (3) electrolysis efficiency of the electrolysis cell and separation efficiency of the ion exchange membrane were significantly decreased by the increases in water flow rate and salt concentration in the feed solution. Later, Hsu (2005) also reported that ORP decreased with increases in water follow rate and free chlorine increased with increases of salt concentration and decrease of water flow rate.

4. The advantages and disadvantages of EO water

The main advantage of EO water is its safety. EO water which is also a strong acid, is different to hydrochloric acid or sulfuric acid in that it is not corrosive to skin, mucous membrane, or organic material. On the other hand, sodium hypochlorite was proved to have a strong toxicity, such as skin irritation, membrane irritation, acute toxicity, and so on (Mori et al., 1997; Sekiya, Ohmori, & Harii, 1997; Shigeto et al., 2000). Currently used hatchery sanitizers

(formaldehyde gas and glutaraldehyde) are noxious to humans and chicks, and may pose a serious health risk (Russell, 2003). Furthermore, the use of formaldehyde gas and glutaraldehyde are gradually being limited because of the adverse effects this chemical has on the environment. Sakurai et al. (2003) also stated that EO water provides a useful means of cleaning and disinfecting digestive endoscopes between patients. It is safe for the human body and for the environment. In addition, the cost of using EO water is much less expensive (5.3 yen/L) compared with glutaraldehyde (1200 yen/L) (Sakurai et al., 2003).

When EO water comes into contact with organic matter, or is diluted by tap water or reverse osmosis (RO) water, it becomes ordinary water again. Thus, it's less adverse impact on the environment as well as users' health. Moreover, compared with other conventional disinfecting techniques, EO water reduces cleaning times, is easy to handle, has very few side effects, and is relative cheap (Tanaka et al., 1999). Chemicals used for cleaning and disinfection are expensive and represent an operating expense for the dairy producer. Once the initial capital investment is made to purchase an EO water generator, the only operating expenses are water, salts and electricity to run the unit (Walker et al., 2005b).

The main disadvantage of EO water is that the solution rapidly loses its antimicrobial activity if EO water is not continuously supplied with H^+, HOCl and Cl_2 by electrolysis (Kiura et al., 2002). EO water is gaining a reputation in various fields as a more capable disinfectant than conventional chemical disinfectants. However, problems, such as chlorine gas emission, metal corrosion, and synthetic resin degradation, due to its strong acidity and free chlorine content have been a matter of concern. Although metal corrosion and synthetic resin degradation occurred, they were not serious on hemodialysis equipment (Tanaka et al., 1999). Ayebah and Hung (2005) also indicated that EO water did not have any adverse effect on stainless steel, it can still be safely used as a sanitizer to inactivate bacteria on food contact surfaces made from stainless steel in food processing. After disinfection, washing food equipment with sterile water can completely avoid metal corrosion. During the EO water generation process, chlorine ions are generated, and thus chlorine gas is emitted. This necessitates the use of standard-type extractor fan.

5. Inactivation of microbes using EO water

As shown in Table 1, many studies have been conducted in evaluating the bactericidal activity of EO water. EO water possess antimicrobial activity on a variety of microorganisms including *Pseudomonas aeruginosa* (Kiura et al., 2002; Vorobjeva et al., 2003), *Staphylococcus aureus* (Park et al., 2002b; Vorobjeva et al., 2003), *S. epidermidis*, *E. coli* O157:H7 (Kim et al., 2000a, 2000b; Park, Hung, & Chung, 2004; Venkitanarayanan et al., 1999b), *Salmonella* Enteritidis (Venkitanarayanan et al., 1999b), *Salmonella* Typhimu-

rium (Fabrizio & Cutter, 2003), *Bacillus cereus* (Len, Hung, Erickson, & Kim, 2000; Sakashita, Iwasawa, & Nakamura, 2002; Vorobjeva et al., 2003), *Listeria monocytogenes* (Fabrizio & Cutter, 2003; Park et al., 2004; Vorobjeva et al., 2003), *Mycobacterium tuberculosis* (Iwasawa & Nakamura, 1993), *Campylobacter jejuni* (Park et al., 2002a), *Enterobacter aerogenes* (Park et al., 2002b) and *Vibrio parahaemolyticus* (Huang et al., 2006a; Kimura et al., 2006). EO water can also reduce germination of many fungal species, such as *Alternaria* spp., *Bortrytis* spp., *Cladosporium* spp., *Colletotrichum* spp., *Curvularia lunata*, *Didymella bryonaie*, *Epicoccum nigrum*, *Fusarium* spp., *Helminthosporium* spp., *Pestalotia* spp., *Phomopsis longicolla*, *Rhodosporidium toruloides*, *Stagonospora nodorum*, *Thielaviopsis basicola*, *Trichoderma spirale*, *Acidovorax avenae* subsp., *Erwinia chrysanthemi*, *Pantoea ananatis*, *Pseudomonas syringae* (Buck, Iersel, Oetting, & Hung, 2002), *Aspergillus* spp. (Buck et al., 2002; Suzuki et al., 2002b), *Botryosphaeria berengeriana* (Al-Haq et al., 2002), *Monilinia fructicola* (Al-Haq et al., 2001; Buck et al., 2002), *Penicillium expansum* (Okull & Laborde, 2004) and *Tilletia indica* (Bonde et al., 1999).

In general, bacteria generally grow in a pH range of 4–9. Aerobic bacteria grow mostly at ORP range +200 to 800 mV, while anaerobic bacteria grow well at −700 to +200 mV. The high ORP in the EO water could cause the modification of metabolic fluxes and ATP production, probably due to the change in the electron flow in cells. Low pH may sensitize the outer membrane of bacterial cells to the entry of HOCl into bacterial cells (McPherson, 1993). HOCl, the most active of the chlorine compounds, appears to kill the microbial cell through inhibiting glucose oxidation by chlorine-oxidizing sulfhydryl groups of certain enzymes important in carbohydrate metabolism. Other modes of chlorine action that have been proposed are: (1) disruption of protein synthesis; (2) oxidative decarboxylation of amino acids to nitrites and aldehydes; (3) reactions with nucleic acids, purines, and pyrimidines; (4) unbalanced metabolism after the destruction of key enzymes; (5) induction of deoxyribonucleic acid (DNA) lesions with the accompanying loss of DNA-transforming ability; (6) inhibition of oxygen uptake and oxidative phosphorylation, coupled with leakage of some macromolecules; (7) formation of toxic *N*-chlorine derivatives of cytosine; and (8) creation of chromosomal aberrations (Marriott & Gravani, 2006).

A theory for inactivation of bacteria based on the high oxidation potential of EO water causing damage of cell membranes was reported by Liao, Chen, and Xiao (2007). The chemical process of oxidation occurs when oxygen contacts with other compounds causing them to lose electrons and further causing the compounds to break down and change functions. In the case of microbes, oxidation could damage cell membranes, create disruption in cell metabolic processes and essentially kill the cell. The bactericidal effects of EO water on *Staphylococcus saprophyticus*, *Micrococcus luteus* and *Bacillus sphaericus* can be seen by

Table 1
A comparison of bactericidal effects on bacterial strains treated with electrolyzed oxidizing water

Bacterial species	Surviving bacterial population after exposing time (mean log CFU/mL)						EO water property				Ref.
	0 s	30 s	1 min	5 min	10 min	15 min	pH	ORP (mV)	Free chlorine (mg/L)	Temperature (°C)	
Gram-negative											
Escherichia coli O157:H7	7.98 ± 0.04	–	–	<1.0	0	0	2.36	1153	86.3	4	Venkitanarayanan et al. (1999b)
Escherichia coli O157:H7	8.04 ± 0.07	–	–	<1.0	0	0	2.37	1155	82.3	23	Venkitanarayanan et al. (1999b)
Salmonella Enteritidis	7.74 ± 0.08	–	–	1.06 ± 0.15	0	0	2.48	1153	83.5	4	Venkitanarayanan et al. (1999b)
Salmonella Enteritidis	7.76 ± 0.08	–	–	<1.0	0	0	2.45	1151	82.0	23	Venkitanarayanan et al. (1999b)
Salmonella Typhimurium	5.20 ± 1.0	–	–	5.13 ± 1.20	3.37 ± 0.70	3.32 ± 0.50	2.30	1155	50	4	Fabrizio and Cutter (2003)
Salmonella Typhimurium	5.11 ± 1.60	–	–	3.46 ± 1.40	0	0	2.60	1150	50	25	Fabrizio and Cutter (2003)
Pseudomonas aeruginosa	8.04 ± 0.07	0	–	–	–	–	2.84	1125	43	23	Vorobjeva et al. (2003)
Escherichia coli	8.21 ± 0.04	0	–	–	–	–	2.84	1125	43	23	Vorobjeva et al. (2003)
Citrobacter freundii	7.63 ± 0.06	0	–	–	–	–	2.84	1125	43	23	Vorobjeva et al. (2003)
Flavobacter sp.	8.12 ± 0.02	0	–	–	–	–	2.84	1125	43	23	Vorobjeva et al. (2003)
Proteus vulgaris	8.01 ± 0.04	0	–	–	–	–	2.84	1125	43	23	Vorobjeva et al. (2003)
Alcaligenes faecalis	7.80 ± 0.03	0	–	–	–	–	2.84	1125	43	23	Vorobjeva et al. (2003)
Aeromonas liquefaciens	7.90 ± 0.04	0	–	–	–	–	2.84	1125	43	23	Vorobjeva et al. (2003)
Campylobacter jejuni	7.42 ± 0.26	<1	–	–	–	–	2.95	1072	25.7	4	Park et al. (2002a)
Campylobacter jejuni	7.47 ± 0.13	<1	–	–	–	–	2.67	1092	53.9	23	Park et al. (2002a)
Campylobacter jejuni	7.42 ± 0.26	0	–	–	–	–	2.67	1092	53.3	4	Park et al. (2002a)
Campylobacter jejuni	7.47 ± 0.13	0	–	–	–	–	2.57	1082	51.6	23	Park et al. (2002a)
Enterococcus faecalis	8.23 ± 0.03	0	–	–	–	–	2.84	1125	43	23	Vorobjeva et al. (2003)
Gram-positive											
Listeria monocytogenes	7.91 ± 0.05	–	–	1.34 ± 0.37	0	0	2.63	1160	43.0	4	Venkitanarayanan et al. (1999b)
Listeria monocytogenes	7.89 ± 0.10	–	–	1.23 ± 0.33	0	0	2.63	1158	48.5	23	Venkitanarayanan et al. (1999b)
Listeria monocytogenes	5.89 ± 0.40	–	–	5.36 ± 0.80	5.12 ± 0.80	4.60 ± 1.10	2.60	1150	50	4	Fabrizio and Cutter (2003)
Listeria monocytogenes	5.10 ± 1.40	–	–	2.66 ± 1.10	0	0	2.60	1150	50	25	Fabrizio and Cutter (2003)
Staphylococcus aureus	8.36 ± 0.08	0	–	–	–	–	2.84	1125	43	23	Vorobjeva et al. (2003)
Staphylococcus aureus	8.03 ± 0.03	0	–	–	–	–	2.53	1178	53.1	23	Park et al. (2002b)
Staphylococcus aureus	8.03 ± 0.03	0	–	–	–	–	2.79	1163	26.9	23	Park et al. (2002b)
Staphylococcus aureus	8.03 ± 0.03	3.92 ± 0.11	–	–	–	–	3.18	1116	11.3	23	Park et al. (2002b)
Bacillus cereus	6.72 ± 0.02	3.76 ± 0.02	–	–	–	–	2.84	1125	43	23	Vorobjeva et al. (2003)
Bacillus cereus (spores)	7.98 ± 0.06	–	0	–	–	–	2.84	1125	43	23	Vorobjeva et al. (2003)
Enterobacter aerogenes	7.98 ± 0.04	0	–	–	–	–	2.53	1178	53.1	23	Park et al. (2002b)
Enterobacter aerogenes	7.98 ± 0.04	0	–	–	–	–	2.79	1163	26.9	23	Park et al. (2002b)
Enterobacter aerogenes	7.98 ± 0.04	0	–	–	–	–	3.18	1116	11.3	23	Park et al. (2002b)

0, Complete inactivation of bacterial culture; –, not measured.

using a scanning electron microscope. The cells treated with electrolyzed acidic water had wrinkled cell wall with round pores in which the cytoplasmic structures were flushed out (Osafune, Ehara, & Ito, 2006).

Little reports on the effects of chlorine, pH and ORP values of the EO water in inactivation of pathogens are available. Kim et al. (2000b) have developed chemically modified water from deionized water with the same properties (i.e., pH, chlorine and ORP) as EO water without using electrolysis. Their results suggested that ORP of EO water might be the primary factor responsible for the bactericidal effect. However, Koseki et al. (2001) noted that the ORP is not the main factor of antimicrobial activity because the higher ORP of ozonated water did not show higher disinfectant effect than lower ORP of EO water. They further defined that free chlorine of EO water, mainly hypochlorous acid (HOCl), produces hydroxyl radical ($\cdot$OH) that acts on microorganisms. Ozone solution produces $\cdot$OH, too. The higher $\cdot$OH produced by higher HOCl concentration in EO water means the better the disinfectant efficacy than ozone solution. Len et al. (2000) reported that the relative concentrations of aqueous molecular chlorine, HOCl, hypochlorite ion (OCl^-) and chlorine gas (Cl_2) were also the factors that accounted for the bactericidal potency. At pH 4, EO water with the maximum concentration of HOCl had the maximum microbicidal activity.

Park et al. (2004) investigated the effects of chlorine and pH on efficacy of EO water for inactivating *E. coli* O157:H7 and *L. monocytogenes*. It was demonstrated that EO water is very effective for inactivating *E. coli* O157:H7 and *L. monocytogenes* in a wide pH range (between 2.6 and 7.0), if sufficient free chlorine (>2 mg/L) is present. For each chlorine content, bactericidal activity and ORP increased with decreasing pH. Based on fluorescent and spectroscopic measurements, Liao et al. (2007) reported that the ORP of EO water could damage the outer and inner membranes of *E. coli* O157:H7. The redox state of the glutathione disulfide–glutathione couple (GSSG/2GSH) can serve as an important indicator of redox environment. There are many redox couples in a cell that work together to maintain the redox environment. The inactivation mechanism hypothesized was that ORP could damage the redox state of GSSG/2GSH and then penetrate the outer and inner membranes of cell, giving rise to the release of intracellular components and finally cause the necrosis of *E. coli* O157:H7. Thus, the antimicrobial effect of EO water derives from the combined action of the hydrogen ion concentration, oxidation–reduction potential and free chlorine.

Storage conditions can affect chemical and physical properties of EO water. When stored under an open, agitated and diffused light condition the EO water had the highest chlorine loss rate. Under open condition, chlorine loss through evaporation followed first-order kinetics. The rate of chlorine loss was increased abound 5-fold with agitation, but it was not significantly affected by diffused light (Len, Hung, & Chung, 2002). EO water exposed to the atmosphere could reduce more chlorine and oxygen than that kept to a closed systems for a longer time (Hsu & Kao, 2004). Fabrizio and Cutter (2003) reported that EO water stored at 4 °C was more stable than stored at 25 °C.

The effectiveness of chlorine as a bactericidal agent is reduced in the presence of organic matter due to the formation of combined available chlorines. At an identical chlorine concentration, the combined available chlorines had much lower bactericidal activity than the free form (Oomori, Oka, Inuta, & Arata, 2000). For practical application, EO water usually must be used in the presence of amino acids or proteins containing materials produce a combined form. Although the electrolyzed solution is not a newly discovered disinfectant, it is important to examine its bactericidal effect on different bacteria (Table 1).

6. Inactivation of blood-virus using EO water

Researchers also indicated that EO water has antiviral potency on blood borne pathogenic viruses including hepatitis B virus (HBV), hepatitis C virus (HCV) (Morita et al., 2000; Sakurai et al., 2003; Tagawa et al., 2000) and human immunodeficiency virus (HIV) (Kakimoto et al., 1997; Kitano et al., 2003; Morita et al., 2000). EO water contained only 4.2 mg/L of free chlorine (pH 2.34, ORP 1053 mV) had a greater efficacy against hepatitis B virus surface antigen (HBsAg) and HIV-1 than sodium hypochlorite (Morita et al., 2000). The possible mechanisms underlying the EO water disinfection against blood-borne viruses might include (1) inactivation of surface protein; (2) destruction of virus envelope; (3) inactivation of viral nucleic acids encoding for enzymes; and (4) destruction of viral RNA (Morita et al., 2000). Hanson, Gor, Jeffries, and Collins, 1989 demonstrated that dried HIV is relatively resistant against disinfectants compared with wet HIV. In an insightful work, Kitano et al. (2003) stated that EO water has an inactivation potential against the infectivity of dried HIV-1. They found that the viral reverse transcript (RT) and the viral RNA in HIV-1 are targets of EO water. Sakurai et al. (2003) reported experiments with HBC and HCV-contaminated endoscopes, and concluded that neither HBV nor HCV was detected after the endoscopes were cleaned manually with a brush and disinfected with EO water. Viral DNA was not detected from any endoscope experimentally contaminated with viral-positive mixed sera (Lee et al., 2004; Tagawa et al., 2000). Thus, EO water directly inactivates viruses and its clinical application is recommended. Effectiveness of EO water in preventing viral infection in the food field needs to be further studied.

7. Inactivation of toxins using EO water

Staphylococcal food poisoning results from the consumption of a food in which enterotoxigenic staphylococci have grown and produced toxins. Within 1–6 h after ingestion of staphylococcal enterotoxin (SEs)-contaminated foods, victims experience nausea, abdominal cramps, vom-

iting, and diarrhea (Archer & Young, 1988; Garthright, Archer, & Kvenberg, 1988). Although EO water has been proved to be effective against *Staphylococcus aureus*, trace amounts of enterotoxin produced by the bacteria may remain active after disinfection. Suzuki, Itakura, Watanabe, and Ohta (2002a) reported that exposure of 70 ng, or 2.6 pmol, of staphylococcal enterotoxin A (SEA) in 25 μL of phosphate buffer saline (PBS) to a 10-fold volume of EO water, or 64.6×10^3-fold molar excess of HOCl in EO water, caused a loss of immuno-reactivity between SEA and a specific anti-SEA antibody. Native PAGE indicated that EO water caused fragmentation of SEA, and amino acid analysis indicated a loss in amino acid content, in particular Met, Tyr, Ile, Asn, and Asp. EO water denatures SEA through an oxidative reaction caused by OH radicals and reactive chlorine. Thus, EO water might be useful as a preventive measure against food-borne disease caused by SEA.

Suzuki et al. (2002b) also reported that EO water could sterilize *Aspergillus parasiticus* and eliminate the mutagenicity of aflatoxin AFB_1 by the OH radical originating from HOCl. Exposing *A. parasiticus* at an initial density of 10^3 spores in 10 μL to a 50-fold volume (500 μL) of EO water containing 390 μmol HOCl for 15 min at room temperature resulted in a complete inhibition of fungal growth. Three nanomoles of AFB_1 showed a high mutagenicity for both *Salmonella* Typhimurium TA98 and TA100 strains, but this mutagenicity was reduced markedly after exposure to 20-fold molar amount of HOCl in the EO water in both TA98 and TA100. However, foods contain compounds such as proteins, lipids, vitamins, minerals, color, etc., and concerning food soundness, it may not necessarily be appropriate to apply EO water to wash food materials.

8. EO water used as a disinfectant in the food industry

8.1. Use of EO water for food processing equipment

EO water has been used as a disinfectant for food processing equipment (Table 2). Venkitanarayanan et al. (1999a) reported EO water could be used as an effective method for eliminating food-borne pathogens on cutting boards. EO water (pH of 2.53, ORP of 1178 mV and chlorine of 53 mg/L) could also reduce *Enterobacter aerogenes* and *S. aureus* on glass, stainless, steel, glazed ceramic tile, unglazed ceramic tile and vitreous china surfaces. Immersion of these surfaces in EO water for 5 min with agitation (50 rpm) reduced populations of *E. aerogenes* and *S. aureus* on the tested surfaces to <1 CFU/cm^2 (Park et al., 2002b). *Listeria monocytogenes* is a food-borne pathogen that can lead to potentially life-threatening listeriosis in high-risk populations. Listeriosis outbreaks have been associated with processed foods and the formation of *L. monocytogenes* biofilms in the processing environment is an important source for secondary contamination (Carpentier & Chassaing, 2004).

Frank and Koffi (1990) and Lee and Frank (1991) earlier reported that *L. monocytogenes* biofilms are resistant to chlorine, acid anionic and quaternary ammonium sanitizers, so that inadequate cleaning and sanitation of food processing surfaces may lead to spread of the pathogen throughout the entire processing plant. Kim et al. (2001) investigated the resistance of *L. monocytogenes* biofilms on stainless steel surfaces to EO water (pH of 2.60, ORP of 1160 mV and chlorine of 56 mg/L) and found that a 300-s treatment on a stainless steel surface, could reduce the *L. monocytogenes* from 1.9×10^{10} CFU/82.5 cm^2 to below detection levels (5 CFU/coupon). However, it took 300 s of exposure to 200 mg/L chlorine solution to achieve the same result. Ayebah et al. (2005) recently inactivated *L. monocytogenes* biofilms on stainless steel surfaces with a combination of ER and EO water. They found that ER water alone did not significantly reduce the *L. monocytogenes* biofilms. Treatment with EO water for only 30–120 s reduced the viable bacteria populations in biofilms by 4.3–5.2 log CFU per coupon (2 by 5 cm), whereas the combined treatment of ER water followed by EO water could produce an additional reduction by 0.3–1.2 log CFU per coupon.

Stainless steel has been the most commonly used material for food contact surfaces in the food industry. Ayebah and Hung (2005) reported that EO water (pH of 2.42, ORP of 1077 mV and free chlorine of 50 mg/L) and modified EO water (pH of 6.12, ORP of 774 mV and free chlorine of 50 mg/L) did not have any adverse effect on stainless steel for a period of 8 days.

The effect of EO water in reducing bacteria in the pipelines of the milking system has been investigated (Walker et al., 2005a, 2005b). A 10 min wash with 60 °C ER water followed by a 10 min wash with 60 °C EO water successfully removed all detectable bacteria from the non-porous milk contact surfaces and ATP residue tests were negative. These results indicated that EO water has the potential to be used as a cleaning and sanitizing agent for cleaning in place (CIP) cleaning of on-farm milking systems.

8.2. Use of EO water for vegetables

Electrolyzed water has been used to inactivate pathogens on fresh produce (Table 3). Izumi (1999) has demonstrated that EO water is usable for cleaning fresh-cut carrots, bell peppers, spinach, Japanese radish and potatoes. The pre-cut produces, treated with EO water (pH 6.8, 20 mg/L free chlorine) by dipping, rinsing or dipping/blowing, showed a bacterial reduction by 0.6–2.6 logs CFU/g. The EO water containing 50 mg/L chlorine had a stronger bactericidal effect than that containing 15 or 30 mg/L chlorine. The treatment did not cause discoloration of fresh-cut produces. Rinsing EO water (50 mg/L) treated fresh-cut produces with fresh water did not increase the bacterial reduction due to the additive effects of the sequential treatment. Koseki et al. (2004b) reported that cucumbers washed with

Table 2
Inactivation of food-borne pathogens on food processing materials by electrolyzed oxidizing water

Processing materials	Immersion condition	Indicator	Effectiveness	EO water property				Ref.
				pH	ORP (mV)	Free chlorine (mg/L)	Temperature (°C)	
Kitchen cutting board	10 min	*Escherichia coli* O157:H7	++	2.50	1163	87	23	Venkitanarayanan et al. (1999a)
Kitchen cutting board	20 min	*Escherichia coli* O157:H7	++	2.56	1165	80	23	Venkitanarayanan et al. (1999a)
Kitchen cutting board	10 min	*Escherichia coli* O157:H7	+++	2.58	1161	87	35	Venkitanarayanan et al. (1999a)
Kitchen cutting board	20 min	*Escherichia coli* O157:H7	+++	2.56	1162	90	35	Venkitanarayanan et al. (1999a)
Kitchen cutting board	5 min	*Escherichia coli* O157:H7	++	2.46	1154	87	45	Venkitanarayanan et al. (1999a)
Kitchen cutting board	10 min	*Escherichia coli* O157:H7	+++	2.51	1157	93	45	Venkitanarayanan et al. (1999a)
Kitchen cutting board	5 min	*Escherichia coli* O157:H7	+++	2.29	1147	45	55	Venkitanarayanan et al. (1999a)
Kitchen cutting board	20 min	*Listeria monocytogenes*	+++	2.50	1156	72	23	Venkitanarayanan et al. (1999a)
Kitchen cutting board	10 min	*Listeria monocytogenes*	++	2.38	1156	66	35	Venkitanarayanan et al. (1999a)
Kitchen cutting board	10 min	*Listeria monocytogenes*	++	2.33	1150	52	45	Venkitanarayanan et al. (1999a)
Glass	5 min	*Enterobacter aerogenes*	++	2.53	1178	53	23	Park et al. (2002a)
Glass	5 min and 50 rpm	*Enterobacter aerogenes*	+++	2.53	1178	53	23	Park et al. (2002a)
Stainless steel	5 min	*Enterobacter aerogenes*	++	2.53	1178	53	23	Park et al. (2002a)
Stainless steel	5 min and 50 rpm	*Enterobacter aerogenes*	+++	2.53	1178	53	23	Park et al. (2002a)
Glazed ceramic tile	5 min	*Enterobacter aerogenes*	++	2.53	1178	53	23	Park et al. (2002a)
Glazed ceramic tile	5 min and 50 rpm	*Enterobacter aerogenes*	+++	2.53	1178	53	23	Park et al. (2002a)
Unglazed ceramic tile	5 min	*Enterobacter aerogenes*	++	2.53	1178	53	23	Park et al. (2002a)
Unglazed ceramic tile	5 min and 50 rpm	*Enterobacter aerogenes*	+++	2.53	1178	53	23	Park et al. (2002a)
Vitreous china	5 min	*Enterobacter aerogenes*	++	2.53	1178	53	23	Park et al. (2002a)
Vitreous china	5 min and 50 rpm	*Enterobacter aerogenes*	+++	2.53	1178	53	23	Park et al. (2002a)
Glass	5 min	*Staphylococcus aureus*	+	2.53	1178	53	23	Park et al. (2002a)
Glass	5 min and 50 rpm	*Staphylococcus aureus*	+++	2.53	1178	53	23	Park et al. (2002a)
Stainless steel	5 min	*Staphylococcus aureus*	+	2.53	1178	53	23	Park et al. (2002a)
Stainless steel	5 min and 50 rpm	*Staphylococcus aureus*	+++	2.53	1178	53	23	Park et al. (2002a)
Glazed ceramic tile	5 min	*Staphylococcus aureus*	+	2.53	1178	53	23	Park et al. (2002a)
Glazed ceramic tile	5 min and 50 rpm	*Staphylococcus aureus*	+++	2.53	1178	53	23	Park et al. (2002a)
Unglazed ceramic tile	5 min	*Staphylococcus aureus*	+	2.53	1178	53	23	Park et al. (2002a)
Unglazed ceramic tile	5 min and 50 rpm	*Staphylococcus aureus*	+++	2.53	1178	53	23	Park et al. (2002a)
Vitreous china	5 min	*Staphylococcus aureus*	+	2.53	1178	53	23	Park et al. (2002a)
Vitreous china	5 min and 50 rpm	*Staphylococcus aureus*	+++	2.53	1178	53	23	Park et al. (2002a)
Stainless steel	0.5 min	*Listeria monocytogenes* biofilms	++	2.40	1163	47	23	Ayebah et al. (2005)
Stainless steel	1 min	*Listeria monocytogenes* biofilms	++	2.40	1163	47	23	Ayebah et al. (2005)
Stainless steel	2 min	*Listeria monocytogenes* biofilms	++	2.40	1163	47	23	Ayebah et al. (2005)
Stainless steel	0.5 min	*Listeria monocytogenes* biofilms	++	2.38	1169	84	23	Ayebah et al. (2005)
Stainless steel	1 min	*Listeria monocytogenes* biofilms	++	2.38	1169	84	23	Ayebah et al. (2005)
Stainless steel	2 min	*Listeria monocytogenes* biofilms	++	2.38	1169	84	23	Ayebah et al. (2005)
Stainless steel	1 min	*Listeria monocytogenes* biofilms	+++	2.6	1160	56	23	Kim et al. (2001)
Stainless steel	5 min	*Listeria monocytogenes* biofilms	+++	2.6	1160	56	23	Kim et al. (2001)

+++, bacterial reduction being more than 6 log CFU/ per unit; ++, bacterial reduction being between 2 and 6 log CFU/ per unit; +, bacterial reduction being less than 2 log CFU/ per unit.

Table 3
Inactivation of food-borne pathogens on vegetables by electrolyzed oxidizing water

Vegetables	Immersion condition	Indicator	Effectiveness	EO water property				Ref.
				pH	ORP (mV)	Free chlorine (mg/L)	Temperature (°C)	
Carrot	EO 4 min	Aerobic bacteria counts	++	6.8	–	20	23	Izumi (1999)
Spinach	EO 4 min	Aerobic bacteria counts	+++	6.8	–	20	23	Izumi (1999)
Bell pepper	EO 4 min	Aerobic bacteria counts	+	6.8	–	20	23	Izumi (1999)
Japanese radish	EO 4 min	Aerobic bacteria counts	+	6.8	–	20	23	Izumi (1999)
Potato	EO 4 min	Aerobic bacteria counts	+	6.8	–	20	23	Izumi (1999)
Cucumber	EO 5 min	Aerobic bacteria counts	++	2.6	1130	32	23	Koseki et al. (2004b)
Cucumber	EO 5 min	Coliform bacteria	+++	2.6	1130	32	23	Koseki et al. (2004b)
Cucumber	EO 5 min	Fungi	+++	2.6	1130	32	23	Koseki et al. (2004b)
Cucumber	EO 5 min+23 °C ER 5 min	Aerobic bacteria counts	+++	2.6	1130	32	23	Koseki et al. (2004b)
Cucumber	EO 5 min+23 °C ER 5 min	Coliform bacteria	+++	2.6	1130	32	23	Koseki et al. (2004b)
Cucumber	EO 5 min+23 °C ER 5 min	Fungi	+++	2.6	1130	32	23	Koseki et al. (2004b)
Lettuce	EO 10 min	Aerobic bacteria counts	+++	2.6	1140	30	23	Koseki et al. (2001)
Lettuce	EO 1 min+23 °C ER 1 min	Aerobic bacteria counts	+++	2.6	1140	30	23	Koseki et al. (2001)
Lettuce	EO 5 min	*Escherichia coli* O157:H7	++	2.6	–	40	20	Koseki et al. (2004c)
Lettuce	EO 5 min+ EO 5 min	*Escherichia coli* O157:H7	++	2.6	–	40	20	Koseki et al. (2004c)
Lettuce	20 °C ER 5 min+ EO 5 min	*Escherichia coli* O157:H7	++	2.6	–	40	20	Koseki et al. (2004c)
Lettuce	EO 5 min	*Salmonella* sp.	++	2.6	–	40	20	Koseki et al. (2004c)
Lettuce	EO 5 min+ EO 5 min	*Salmonella* sp.	++	2.6	–	40	20	Koseki et al. (2004c)
Lettuce	20 °C ER 5 min+ EO 5 min	*Salmonella* sp.	++	2.6	–	40	20	Koseki et al. (2004c)
Lettuce	EO 1 min	*Escherichia coli* O157:H7	+	2.6	–	40	4	Koseki et al. (2004c)
Lettuce	EO 5 min	*Escherichia coli* O157:H7	++	2.6	–	40	4	Koseki et al. (2004c)
Lettuce	1 min EO	*Escherichia coli* O157:H7	+	2.6	–	40	20	Koseki et al. (2004c)
Lettuce	5 min EO	*Escherichia coli* O157:H7	++	2.6	–	40	20	Koseki et al. (2004c)
Lettuce	1 min EO	*Escherichia coli* O157:H7	+++	2.6	–	40	50	Koseki et al. (2004c)
Lettuce	5 min EO	*Escherichia coli* O157:H7	++++	2.6	–	40	50	Koseki et al. (2004c)
Lettuce	1 min EO	*Salmonella* sp.	+	2.6	–	40	4	Koseki et al. (2004c)
Lettuce	5 min EO	*Salmonella* sp.	++	2.6	–	40	4	Koseki et al. (2004c)
Lettuce	1 min EO	*Salmonella* sp.	+	2.6	–	40	20	Koseki et al. (2004c)
Lettuce	5 min EO	*Salmonella* sp.	++	2.6	–	40	20	Koseki et al. (2004c)
Lettuce	1 min EO	*Salmonella* sp.	+++	2.6	–	40	50	Koseki et al. (2004c)
Lettuce	5 min EO	*Salmonella* sp.	+++	2.6	–	40	50	Koseki et al. (2004c)
Lettuce	20 °C ER 1 min +1 or 5 min EO	*Escherichia coli* O157:H7	++	2.6	–	40	4	Koseki et al. (2004c)
Lettuce	20 °C ER 5 min +1 or 5 min EO	*Escherichia coli* O157:H7	++	2.6	–	40	4	Koseki et al. (2004c)
Lettuce	50 °C ER 1 min +1 or 5 min EO	*Escherichia coli* O157:H7	+++	2.6	–	40	4	Koseki et al. (2004c)
Lettuce	50 °C ER 5 min +1 or 5 min EO	*Escherichia coli* O157:H7	+++	2.6	–	40	4	Koseki et al. (2004c)
Lettuce	20 °C ER 1 min +1 or 5 min EO	*Salmonella* sp.	++	2.6	–	40	4	Koseki et al. (2004c)
Lettuce	20 °C ER 5 min +1 or 5 min EO	*Salmonella* sp.	++	2.6	–	40	4	Koseki et al. (2004c)
Lettuce	50 °C ER 1 min +1 or 5 min EO	*Salmonella* sp.	+++	2.6	–	40	4	Koseki et al. (2004c)
Lettuce	50 °C ER 5 min +1 or 5 min EO	*Salmonella* sp.	+++	2.6	–	40	4	Koseki et al. (2004c)
Lettuce	1 min EO	*Escherichia coli* O157:H7	++++	2.5	1030	45	22	Park et al. (2001)
Lettuce	3 min EO	*Escherichia coli* O157:H7	++++	2.5	1030	45	22	Park et al. (2001)
Lettuce	1 min EO	*Listeria monocytogenes*	+++	2.5	1030	45	22	Park et al. (2001)
Lettuce	3 min EO	*Listeria monocytogenes*	++++	2.5	1030	45	22	Park et al. (2001)
Alfalfa seeds	3 hr	*Salmonella* sp.	++	2.4	1081	84	23	Kim et al. (2003)

Table 3 (continued)

Vegetables	Immersion condition	Indicator	Effectiveness	EO water property			Temperature (°C)	Ref.
				pH	ORP (mV)	Free chlorine (mg/L)		
Alfalfa seeds	3 hr	Non-Salmonella microflora	++	2.4	1081	84	23	Kim et al. (2003)
Alfalfa sprouts	10 min EO & sonication	Salmonella sp.	++	2.4	1081	84	23	Kim et al. (2003)
Alfalfa sprouts	10 min EO & sonication	Non-Salmonella microflora	+	2.4	1081	84	23	Kim et al. (2003)
Alfalfa sprouts	10 min EO & seed coat removal	Salmonella sp.	++	2.4	1081	84	23	Kim et al. (2003)
Alfalfa sprouts	10 min EO & seed coat removal	Non-Salmonella microflora	++	2.4	1081	84	23	Kim et al. (2003)
Alfalfa sprouts	10 min EO & sonication & seed coat removal	Salmonella sp.	+++	2.4	1081	84	23	Kim et al. (2003)
Alfalfa sprouts	10 min EO & sonication & seed coat removal	Non-Salmonella microflora	++	2.4	1081	84	23	Kim et al. (2003)
Alfalfa seeds	15 min EO	Salmonella sp.	++	2.5	1079	70	23	Stan and Daeschel (2003)
Alfalfa seeds	60 min EO	Salmonella sp.	++	2.6	1076	66.8	23	Stan and Daeschel (2003)

++++, bacterial reduction being more than 4 log CFU/ per unit; +++, bacterial reduction being between 2 and 4 CFU/ per unit; ++, bacterial reduction being between 1 and 2 CFU/ per unit; +, bacterial reduction being less than 1 log CFU/ per unit. −, not measured.

ER water (pH of 11.3, ORP of −870 mV) for 5 min and then soaked in EO water (pH of 2.6, ORP of 1130 mV and free chlorine of 30 mg/L) for 5 min showed a reduction in aerobic mesophiles. This treatment had at least 2 log CFU per cucumber greater reduction than that only soaked in EO water (30 mg/L free chlorine), ozonated water (5 mg/L ozone) or sodium hypochlorite solution (NaOCl, 150 mg/L free chlorine) for 10 min. In studies on sequential wash treatment, Koseki et al. (2001) also found that a 2-log CFU/g reduction in aerobic bacteria counts for both the lettuce treated with ER water for 1 min followed by the treatment with EO water for 1 min and the lettuce treated with acidic EO water alone for 10 min; however, repeated EO water treatment did not show a significant increase of bacterial reduction. Koseki et al. (2004c) used mildly heated (50 °C) ER water to treat lettuce for 5 min, and then used chilled (4 °C) EO water to treat for 1 or 5 min. They found the treatment could reduce both E. coli O157:H7 and Salmonella at a level of 3–4 log CFU/g. Wang, Feng, and Luo (2004) washed fresh-cut cilantro with ozonated water for 5 min followed with a EO water (pH of 2.45, ORP of 1130 mV and free chlorine of 16.8 mg/L) for 5 min and found that the sequential wash is effective in reducing initial microbial count and slowing microbial growth during storage.

Lettuce with smooth surfaces have been used for the investigation of the effectiveness of EO water on bacterial reduction. Park et al. (2001) observed that shaking lettuce with EO water (45 mg/L free chlorine) at 100 rpm for 3 min significantly decreased mean populations of E. coli O157:H7 and L. monocytogenes by 2.41 and 2.65 log CFU per lettuce leaf, respectively, when compared with sterile H_2O treatment. The result was in agreement with that of Izumi (1999) who pointed out that EO water (50 mg/L of free chlorine) treatment of shredded lettuce did not significantly affect the quality characteristics such as color and general appearance. Yang et al. (2003) suggested that fresh-cut lettuce dipped in EO water (pH 7) containing 300 mg/L of free chlorine for 5 min could not only keep the best visual quality but also achieve a 2-log CFU/g reduction for S. Typhimurium, E. coli O157:H7 and L. monocytogenes.

Koseki and Itoh (2001) suggested that the best temperature for distribution of fresh-cut vegetables with reduced microbial population is 1 °C. Ice is an inexpensive material for preserving fresh produces and fish. Koseki, Fujiwara, and Itoh (2002) treated lettuce with frozen EO water (pH of 2.5, ORP of 1148 mV and free chlorine of 20.5 mg/L) and stored in a styrene-foam container for 24 h. The results indicated that a 1.5-log CFU/g aerobic bacteria counts reduction on lettuce was due to an increased chlorine gas concentration from frozen EO water. In order to check the effectiveness of Cl_2 concentration and volume or weight ratio of vegetables to frozen EO water, Koseki, Isobe, and Itoh (2004a) prepared a EO-ice by freezing EO water at −40 °C. The EO water with 20, 50, 100 and 200 mg/L of free chlorine could generate ice with 30, 70, 150 and

240 mg/L of Cl_2, respectively. EO-ice generating 70–240 mg/L Cl_2 significantly reduced *L. monocytogenes* by 1.5 log CFU/g during 24 h storage. EO-ice generating 70–150 mg/L of Cl_2 reduced *E. coli* O157:H7 cell counts by 2.0 log CFU/g. Although higher concentration with 240 mg/L of Cl_2 showed a significantly higher reduction of *E. coli* O157:H7 by 2.5 log CFU/g, accompanied by physiological disorder resembling leaf burn. The weight ratio of EO-ice to lettuce was >10. Chlorine at a level below 150 mg/L did not affect the surface color of the lettuce.

Sprouts have been associated with a number of food-borne illnesses in recent years. *E. coli* O157:H7, *Salmonella* spp. and *B. cereus* have been responsible for several sprout-associated outbreaks worldwide (Taormina, Beuchat, & Slusker, 1999). Sprouts are produced under warm and humid condition, pathogens can grow rapidly during seed germination increasing the likelihood of infections. Beuchat, Ward, and Pettigrew (2001) reported populations of *Salmonella* exceeding 10^6 CFU/g could occur on alfalfa sprouts produced from contaminated seeds. Although the use of 20,000 mg/L $Ca(OCl)_2$ for treatment of seeds intended for sprout production has been recommended (NACMCF, 1999), the use of high concentrations of $Ca(OCl)_2$ both generated worker safety concerns and significantly reduced seed germination rates (70% versus 90–96%) (Kim et al., 2003). Studies have demonstrated that 64.5 mg/L free chlorine in EO water treatment reduced *E. coli* O157:H7 population on alfalfa sprouts (initial population was about 6 log CFU/g) by 1.05 log CFU/g (91.1%) for 2 min treatment, while the reduction was by 2.72 log CFU/g (99.8%) for 64 min treatment. EO water treatment did not cause any visible damage to the sprouts (Sharma & Demirci, 2003). Kim et al. (2003) reported that treatment of seeds with 20,000 mg/L $Ca(OCl)_2$ reduced the population of *Salmonella* and non-*salmonella* to undetectable levels on culture media, but an amount >6 log CFU/g of *Salmonella* was still recovered from sprouts generated from these seeds. However, the combination of EO water (84 mg/L free chlorine) and sonication treatment had a better reduction on *Salmonella* and non-*salmonella* populations than that by using EO water alone. Removal of seed coats by sonication might have detached cells that were attached or entrapped in sprouts, thus making the pathogen more susceptible to the EO water. The combined treatment achieved 2.3 and 1.5 log CFU/g greater reductions than EO water alone in populations of *Salmonella* and non-*salmonella* microflora, respectively (Kim et al., 2003).

8.3. Use of EO water for fruits

Postharvest decay of fruits causes economic loss to the fruit industry. In studies on surface sterilization of fruits, Al-Haq et al. (2001) found that EO water could prevent peach from decay and it could be used as an important alternative to liquid sterilants. Al-Haq et al. (2002) later found that EO water immediately reacted with *Botryosp-haeria berengeriana* that presented on the first few layers of the pear surface and could not control growth of bacteria that entered into the fruit deeper than 2 mm. No chlorine-induced phytotoxicity on the treated fruit was observed. Both EO water containing 200 and 444 mg/L free chlorine significantly reduce the populations of *E. coli* O157:H7, *S. enteritidis* and *L. monocytogenes* on the surfaces of tomatoes without affecting sensory quality (Bari et al., 2003; Deza et al., 2003).

Patulin is a mycotoxin mainly found in apples and their products that are contaminated with the common storage-rot fungus *Penicillium expansum* (Brian, Elson, & Lowe, 1956; Harwig, Chen, Kennedy, & Scott, 1973). The uses of 100% and 50% EO water containing 60 mg/L free chlorine could decrease *P. expansum* viable spore populations by greater than 4 and 2 log units of aqueous suspension and wounded apples (Okull & Laborde, 2004). EO water did not control brown rot in wound-inoculated fruits, but reduced disease incidence. In contrast to the present results for smooth fruits, on treatment of the surface of the strawberry with 30 mg/L free chlorine EO water and 150 mg/L NaOCl, aerobic mesophiles were reduced by less than 1 log CFU per strawberry after washing in ER water (pH of 11.3, ORP of −870 mV) for 5 min and then with EO water (pH of 2.6, ORP of 1130 mV and free chlorine of 30 mg/L) for 5 min, EO water (30 mg/L free chlorine), ozonated water (5 mg/L ozone) and sodium hypochlorite solution (NaOCl, 150 mg/L free chlorine) for 10 min, respectively. These results can be attributed to the surface structure of the strawberry fruit. There are many achenes (seeds) that render its surface structure uneven and complex (Koseki et al., 2004b). These studies showed that the efficacy of EO water as a sanitizing agent was dependent on the surface structure of fruit treated.

8.4. Use of EO water for poultry and meat

Egg shell can serve as a vehicle for transmission of human pathogens. Due to the fecal matter in the nesting place, the wash water during manipulation, or during packaging process, the shell may become contaminated with *E. coli* O157:H7, *Salmonella* sp., *L. monocytogenes* and *Yersinia enterocolitica* (Gabriela, Maria, Lidia, & Ana, 2000; Moore & Madden, 1993; Schoeni & Doyle, 1994). Elimination of pathogens in hatchery facilities has been usually done by applying of formaldehyde and glutaraldehyde gas or fogging hydrogen peroxide. However, these disinfectants may pose high risk for human and chick health. Russell (2003) found that EO water (pH of 2.1, ORP of 1150 mV and free chlorine of 8 mg/L) with an electrostatic spraying system could completely eliminate *S.* Typhimurium, *S. aureus* and *L. monocytogenes* on egg shells.

Efficacy of EO water in reducing pathogens on poultry has been investigated in recent years (Table 4). Park et al. (2002a) reported that for chicken wings (50 ± 5 g) inoculated with *Campylobacter jejuni*, soaking in EO water (pH of 2.57, ORP of 1082 mV and free chlorine of

Table 4
Inactivation of food-borne pathogens on poultry and meat by electrolyzed oxidizing water

Materials	Immersion condition	Indicator	Effectiveness	EO water property				Ref.
				pH	ORP (mV)	Free chlorine (mg/L)	Temperature (°C)	
Chicken wing	10 min EO	*Campylobacter jejuni*	+++	2.5	1082	51.6	23	Park et al. (2002a)
Chicken wing	30 min EO	*Campylobacter jejuni*	+++	2.5	1082	51.6	23	Park et al. (2002a)
Chicken wing	10 min EO	*Campylobacter jejuni*	+++	2.6	1092	53.3	4	Park et al. (2002a)
Chicken wing	30 min EO	*Campylobacter jejuni*	+++	2.6	1092	53.3	4	Park et al. (2002a)
Broiler carcasses	45 min EO	Aerobic bacteria counts	++	2.6	1150	50	4	Fabrizio et al. (2002)
Broiler carcasses	45 min EO	*Salmonella* Typhimurium	+	2.6	1150	50	4	Fabrizio et al. (2002)
Broiler carcasses	45 min EO	*Escherichia coli*	++	2.6	1150	50	4	Fabrizio et al. (2002)
Broiler carcasses	45 min EO	Coliform bacteria	++	2.6	1150	50	4	Fabrizio et al. (2002)
Chicken carcasses	40 min EO	*Campylobacter jejuni*	+++	2.8	1165	39.5	23	Kim et al. (2005)
Pork belly	15 s spray with EO	Aerobic bacteria counts	++	2.6	1150	50	23	Fabrizio and Cutter (2004)
Pork belly	15 s spray with EO	*Escherichia coli*	++	2.6	1150	50	23	Fabrizio and Cutter (2004)
Pork belly	15 s spray with EO	Coliform bacteria	++	2.6	1150	50	23	Fabrizio and Cutter (2004)
Pork belly	15 s spray with EO	*Salmonella* Typhimurium	++	2.6	1150	50	23	Fabrizio and Cutter (2004)
Pork belly	15 s spray with EO	*Listeria monocytogenes*	++	2.6	1150	50	23	Fabrizio and Cutter (2004)
Pork belly	15 s spray with EO	*Campylobacter coli*	++	2.6	1150	50	23	Fabrizio and Cutter (2004)
Cattle hide	10 s spray with ER & 10 s spray with EO	Aerobic bacteria counts	+++	2.4	–	70	60	Bosilevac et al. (2005)
Cattle hide	10 s spray with ER & 10 s spray with EO	*Enterobacteriaceae* counts	++++	2.4	–	70	60	Bosilevac et al. (2005)
Frankfurter	15 min EO	*Listeria monocytogenes*	++	2.3	1150	45	25	Fabrizio and Cutter (2005)
Frankfurter	15 min spray with EO	Aerobic bacteria counts	+	2.3	1150	45	25	Fabrizio and Cutter (2005)
Frankfurter	15 min spray with EO	*Listeria monocytogenes*	+	2.3	1150	45	25	Fabrizio and Cutter (2005)
Ham	15 min spray with EO	*Listeria monocytogenes*	+	2.3	1150	45	25	Fabrizio and Cutter (2005)

++++, bacterial reduction being more than 4 log CFU/ per unit; +++, bacterial reduction being between 2 and 4 CFU/ per unit; ++, bacterial reduction being between 1 and 2 CFU/ per unit; +, bacterial reduction being less than 1 log CFU/ per unit. –, not measured.

Table 5
Inactivation of food-borne pathogens on seafood fields by electrolyzed oxidizing water

Materials	Immersion condition	Indicator	Effectiveness	EO water property				Ref.
				pH	ORP (mV)	Free chlorine (mg/L)	Temperature (°C)	
Salmon fillet	64 min EO	*Escherichia coli*	+	2.6	1150	90	22	Ozer and Demirci (2006)
Salmon fillet	64 min EO	*Escherichia coli*	++	2.6	1150	90	35	Ozer and Demirci (2006)
Salmon fillet	64 min EO	*Listeria monocytogenes*	+	2.6	1150	90	22	Ozer and Demirci (2006)
Salmon fillet	64 min EO	*Listeria monocytogenes*	++	2.6	1150	90	35	Ozer and Demirci (2006)
Tilapia	1 min EO	*Escherichia coli*	+	2.4	1159	120	23	Huang et al. (2006a)
Tilapia	5 min EO	*Escherichia coli*	++	2.4	1159	120	23	Huang et al. (2006a)
Tilapia	10 min EO	*Escherichia coli*	++	2.4	1159	120	23	Huang et al. (2006a)
Tilapia	1 min EO	*Vibrio parahaemolyticus*	++	2.4	1159	120	23	Huang et al. (2006a)
Tilapia	5 min EO	*Vibrio parahaemolyticus*	++	2.4	1159	120	23	Huang et al. (2006a)
Tilapia	10 min EO	*Vibrio parahaemolyticus*	++	2.4	1159	120	23	Huang et al. (2006a)
Dirty fish retailer in fish market	1 min EO	Aerobic bacteria counts	++++	2.2	1145	200	23	Huang et al. (2006a)
Dirty fish retailer in fish market	1 min EO	Aerobic bacteria counts	++	2.5	1120	100	23	Huang et al. (2006a)
Dirty fish retailer in fish market	5 min EO	Aerobic bacteria counts	+++	2.5	1120	100	23	Huang et al. (2006a)
Dirty fish retailer in fish market	10 min EO	Aerobic bacteria counts	+++	2.5	1120	100	23	Huang et al. (2006a)
Dirty fish retailer in fish market	1 min EO	Aerobic bacteria counts	++	2.7	1090	50	23	Huang et al. (2006a)
Dirty fish retailer in fish market	5 min EO	Aerobic bacteria counts	++	2.7	1090	50	23	Huang et al. (2006a)
Dirty fish retailer in fish market	10 min EO	Aerobic bacteria counts	++	2.7	1090	50	23	Huang et al. (2006a)
Tuna fillet	5 min EO (150 rpm)	Aerobic bacteria counts	++	2.5	1105	50	23	Huang et al. (2006b)
Tuna fillet	5 min EO (150 rpm) & CO	Aerobic bacteria counts	+	2.5	1105	50	23	Huang et al. (2006b)
Tuna fillet	5 min EO (150 rpm)	Aerobic bacteria counts	++	2.2	1135	100	23	Huang et al. (2006b)
Tuna fillet	5 min EO (150 rpm) & CO	Aerobic bacteria counts	++	2.2	1135	100	23	Huang et al. (2006b)
Stainless steel containing seafood residue	5 min EO	*Listeria monocytogenes*	+++	2.5	1150	50	23	Liu et al. (2006b)
Ceramic title containing seafood residue	5 min EO	*Listeria monocytogenes*	+++	2.5	1150	50	23	Liu et al. (2006b)
Floor tile containing seafood residue	5 min EO	*Listeria monocytogenes*	++	2.5	1150	50	23	Liu et al. (2006b)

Table 5 (continued)

Materials	Immersion condition	Indicator	Effectiveness	EO water property				Ref.
				pH	ORP (mV)	Free chlorine (mg/L)	Temperature (°C)	
Natural rubber latex glove containing seafood residue	5 min EO	Listeria monocytogenes	+++	2.6	1125	40	23	Liu and Su (2006b)
Natural latex glove containing seafood residue	5 min EO	Listeria monocytogenes	++	2.6	1125	40	23	Liu and Su (2006b)
Nitrile containing seafood residue	5 min EO	Listeria monocytogenes	++	2.6	1125	40	23	Liu and Su (2006b)
Latex (disposable) containing seafood residue	5 min EO	Listeria monocytogenes	+++	2.6	1125	40	23	Liu and Su (2006b)
Nitrile (disposable) containing seafood residue	5 min EO	Listeria monocytogenes	+++	2.6	1125	40	23	Liu and Su (2006b)
Above 5 clean food processing gloves	5 min EO	Listeria monocytogenes	++++	2.6	1125	40	23	Liu and Su (2006b)

++++, bacterial reduction being more than 4 log CFU/ per unit; +++, bacterial reduction being between 2 and 4 CFU/ per unit; ++, bacterial reduction being between 1 and 2 CFU/ per unit; +, bacterial reduction being less than 1 log CFU/ per unit.

50 mg/L) with 100 rpm agitation for 30 min has achieved reduction by 3 log CFU/g. Since pathogens were attached to a water-skin interfaces and further entrapped in folds, crevices and follicles, no viable cell of C. jejuni was recovered in EO water after treatment. Kim, Hung, and Russell (2005) recommended to spray-wash chicken with ER water before defeathering and evisceration to reduce the potential cross-contamination. However, combining immersion with spray-washing did not significantly improve the bactericidal effect of EO water as compared to the immersion-only treatment. Fabrizio et al. (2002) reported that spray-washing with EO water, ozone, 2% acetic acid (AA) or 10% trisodium phosphate (TSP) did not show any significant microbicidal effectiveness. However, spray-washing with ER water followed by immersion in EO water had a better effectiveness than spraying with AA and TSP followed by immersion in chlorine solution at the end of a 7-day refrigerated storage.

Fabrizio and Cutter (2004) had recently examined the spray-washing with EO water for 15 s to disinfect pork bellies inoculated with feces containing L. monocytogenes, S. Typhimurium and Campylobacter coli. This study demonstrated that a 15-s spraying with EO water (pH of 2.4, ORP of 1160 mV and free chlorine of 50 mg/L) had the ability to reduce the populations of L. monocytogenes, S. Typhimurium and C. coli (1.23, 1.67 and 1.81, respectively) on the pork surfaces and inferred that longer contact times might strengthen the disinfection effectiveness. For sterilizing hides of cattle before slaughtering, Bosilevac, Shackelford, Brichta, and Koohmaraie (2005) reported that sequentially applied ER water and EO water containing 70 mg/L free chlorine at 60 °C for a 10-s spraying could reduce aerobic bacteria counts by 3.5 log CFU/100 cm^2 and reduced Enterobacteriaceae counts by 4.3 log CFU/ 100 cm^2. Recently, Fabrizio and Cutter (2005) dipped or sprayed frankfurters and ham inoculated with L. monocytogenes with EO water (pH of 2.3, ORP of 1150 mV and free chlorine of 45 mg/L) and/or ER water for 30 min. No significant difference ($p < 0.05$) between treatments on Hunter L*, a*, b* values for frankfurters and ham at the end of 7 days storage at 4 °C was found. The results indicated that EO water has no detrimental "bleaching" effects on the surface of tested read-to-eat meats.

8.5. Use of EO water for seafood

Using EO water for inactivating bacteria in raw seafood have been reported (Table 5). Ozer and Demirci (2006) found that treating raw salmon with EO water (pH of 2.6, ORP of 1150 mV and free chlorine of 90 mg/L) at 35 °C for 64 min resulted in a 1.07 log CFU/g (91.1%) and 1.12 log CFU/g (92.3%) reduction in E. coli O157:H7 and L. monocytogenes, respectively. Recently, Liu and Su (2006) stated that gloves used in handling food for protection of the worker and seller could become a carrier of pathogens through the contact of raw materials or contaminated surfaces. However, applications of EO water follow-

ing a thorough cleaning greatly reduced *L. monocytogenes* population on gloves and seafood processing plants. Soaking inoculated gloves in EO water (pH of 2.6, ORP of 1125 mV and free chlorine of 40 mg/L) at room temperature for 5 min completely eliminated *L. monocytogenes* on gloves ($>4.46 \log CFU/cm^2$) (Liu & Su, 2006). The treatment by immersion in EO water containing 50 mg/L chlorine for 5 min significantly reduced *L. monocytogenes* on tested surfaces (3.73 $\log/25 cm^2$ on stainless steel sheet, 4.24 $\log/25 cm^2$ on ceramic tile and 1.52 $\log/25 cm^2$ on floor tile) (Liu, Duan, & Su, 2006). Huang et al. (2006a) also reported that EO water was a very effective sanitizer used for cleaning fish contacting surfaces in traditional grocery stores and fish markets, so that secondary bacterial contamination could be prevented. EO water was especially effective in reducing the population of *E. coli* and *V. parahaemolyticus* contamination on tilapia.

In order to prolong the shelf life of yellow-fin tuna (*Thunnus albacares*) during refrigerated and frozen storage, combination of EO water and CO gas were applied. Huang, Shiau, Hung, and Hwang (2006b) reported that tuna treated with a combination of EO water containing 100 mg/L chlorine and CO gas could immediately result in the lowest APC. EO water containing 50 mg/L or 100 mg/L chlorine combined with CO gas treatment in tuna fish steak would be an effective method for enhancing the hygienic quality and freshness for tuna meat and extending refrigerated storage time. The efficiency of EO water on the growth and toxicity of the dinoflagellates *Alexandrium minutum*, *Alexandrium catenella* and *Gymnodinium catenatum* has been studied in our laboratory. It was found that EO water very effectively killed toxic dinoflagillates and destroyed toxicity.

9. Conclusions

Since EO water is considered to be a solution containing HOCl, the application of EO water can be fitted into the regulations for hypochlorous (HOCl). In 2002, Japan had officially approved EO water as a food additive (Yoshida, Achiwa, & Katayose, 2004). Electrolyzed water generator has also been approved for applications in the food industry by the US Environmental Protection Agency (EPA) (Park et al., 2002b).

Although EO water has advantages as a disinfectant for use in many food products, relevant topics in EO water deserve future research. These may include the methods for expanding the usages of EO water in food processing plant and the application in HACCP and SSOP systems. Since bactericidal effects of the EO water may be reduced in the presence of organic matter due to the formation of monochloramines, techniques to avoid these matters need to be researched. Furthermore, the sensory characteristics of food processed may be affected by degradation of contaminants in the food during the application of EO water need to be further studied.

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