

# Application of Electrolyzed Water as Food Sanitizer

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**Abstract**—Uncontrolled growth of microorganism in foodstuff pose a severe challenge to the food industry, as it could lead to food spoilage, or even foodborne disease if the microorganism is pathogenic. To deal with these threats, sanitizers have been widely applied in food industry. During the last two decades, Electrolyzed Water (EW) has been found to be a promising new sanitizer for food industry, as it is more environmentally friendly compared to conventional chlorine-based disinfectants. This paper reviews the recent progress on the application of EW as a food sanitizer. EW was produced by the electrolysis of diluted NaCl (or HCl) solution and could be classified into several subgroups (acid EW, slightly EW, neutral EW, alkaline EW, low concentration EW) based on their pH and available chlorine concentration. The efficacy of using EW to inhibit the growth of several most important microorganism of food safety concern were proven by numerous studies. Besides, the application of EW has been seldomly associated with detrimental effects on the nutritional and sensory proprieties of food. However, its antimicrobial potency was affected by factors such as pH, temperature, storage time, and organic matters.

**Index Terms**—foodborne diseases, food safety, sanitizer, microbiology, electrolyzed water, fresh produce

## I. INTRODUCTION

Despite the advance of personal hygiene, food production, processing and consumer awareness, foodborne diseases remains to be the top concern of food industry. According to the estimate of the Centers for Disease Control and Prevention (CDC) of the United States, 48 million are affected, 128 000 are hospitalized, and 3 000 people die from foodborne illness each year in the United States, and the total cost of foodborne illness is 15.6 billion per year (<https://www.cdc.gov/foodborneburden/estimates-overview.html>). In general, fresh produce is the most common food type lead to foodborne illness. Other than foodborne disease, another big concern for food production is food spoilage. Based on the analysis from the Food and Agriculture Organization of the United Nations, one third of the food produced globally is lost or goes into waste, which accounts to about 1.3 billion tons of food per year. (<http://www.fao.org/food-loss-and-food-waste/en/>).

Foodborne diseases and food spoilage are caused, in most of the cases, by the undesired growth of foodborne

microorganism. To ensure the safety of food and reduce food spoilage, food industries use sanitizers to inhibit microorganisms' growth or to kill proliferating microbes in foods. Currently, the most commonly applied sanitizers by food industry include sodium hypochlorite, iodine, quaternary ammonium compounds, peroxyacetic acid, ozone, hydrogen peroxide, phosphoric acid, potassium hydroxide and chlorine dioxide. [1], [2]

However, despite their wide range of applications, conventional sanitizers all suffer from several limitations. For example, even though being cheap, easily available, and effective, sodium hypochlorite, the current most widely used sanitizer, is suspected to have residual toxicity as well as a pungent odor, corrosive properties, and a cause of skin irritation. [3]-[5] Hence, the food industry is in constant search for new sanitizing agents and techniques that are more potent, safer, and more environmentally friendly. Recently, scientists are investigating the potential of Electrolyzed Water (EW) as a disinfectant to improve food safety because conventional methods have lower acceptability due to consumer perception. EW is considered environmental-friendly sanitizer because it is generated from dilute salt and water, cause less corrosion to surface of production plant and eliminate the effect to human body. [6]-[8]

This paper serves as a systematic review on the recent progress of the application of EW as a sanitizer in food industry. It reviewed the efficacy of EW on major foodborne pathogens and foodborne spoilage microorganisms, the antimicrobial mechanism of EW on microorganisms, factors that affect the antimicrobial efficacy of EW application and the effect of EW on food quality.

## II. ELECTROLYZED WATER

### A. Apparatus Design and Working Mechanism

EW was first invented in Russia in the 1950s, yet its sanitizing potential was not recognized until Japanese scientists used it for water decontamination, water regeneration and disinfection since 1980. [9] EW is referred to any solution produced by the electrolysis of water containing an electrolyte, such as sodium chloride (NaCl), potassium chloride (KCl) or dilute hydrochloric acid, as defined by the Japan Electrolyze Water Association (<https://jewa.org/information/>). In most of the cases, the electrolysis process takes place in a standard

electrolysis chamber, in which the anode and the cathode are separated by a membrane, as shown in Fig. 1. During electrolysis, salt (NaCl) dissolves in water,  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{H}^+$  and  $\text{OH}^-$  are produced in the solution. Negatively charged  $\text{Cl}^-$  and  $\text{OH}^-$  move to anode and positively charged  $\text{Na}^+$  and  $\text{H}^+$  move to cathode. Hypochlorous acid (HOCl),  $-\text{OCl}$ ,  $\text{HCl}$ ,  $\text{O}_2$  and  $\text{Cl}_2$  will be generated in anode and  $\text{NaOH}$  and  $\text{H}_2$  will be generated in cathode. [10] After electrolysis, Acidic Electrolyzed Water (AEW) that has sanitizing and deodorizing ability due to low pH, high ORP and presence of HOCl and other chloride compounds ( $\text{OCl}$ ,  $\text{HCl}$ , and  $\text{Cl}_2$ ) will be produced at the anode. Alkaline Electrolyzed Water (AIEW) that has strong cleansing effect against larger compounds such as protein, fat and oil, is produced at the cathode, together with  $\text{NaOH}$  and  $\text{H}_2$ . [10], [11]

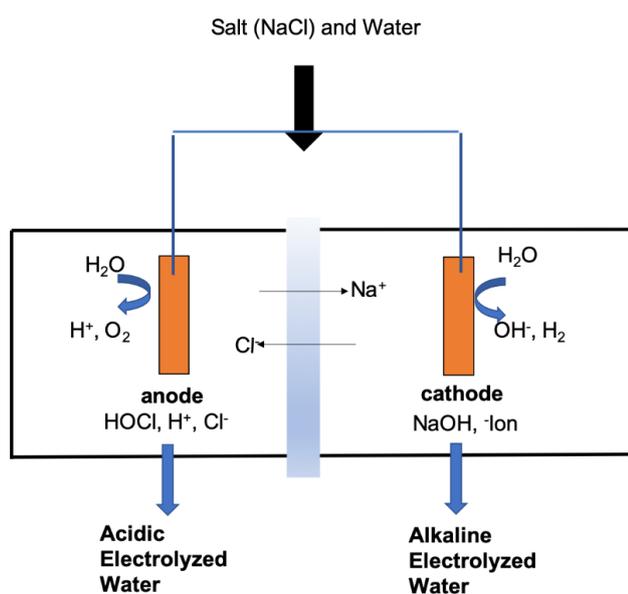


Figure 1. Schematic illustration of the production of electrolyzed water in a standard electrolysis chamber.

### B. Characterization and Classification of EW

In general, EW is characterized by its three most important physical-chemical properties, i.e., Available Chlorine Concentration (ACC), also called Free Chlorine Concentration (FCC) Oxidation Reduction Potential (ORP), and pH values.

ACC describes the amount of chlorines that are freely available to the sanitized objects. In an EW solution, FCC is the sum of the concentrations of freely dissolved chlorine gas ( $\text{Cl}_2$ ), the undissociated hypochlorous acid (HClO) and the hypochlorite anion ( $\text{ClO}^-$ ), and HClO is considered as the effective antimicrobial substance due to its ability to cause protein deterioration and enzyme inactivation. [12]-[15] In addition, HClO is most abundant in EW and is 80 times more effective against *E. coli* at an equivalent concentration of  $\text{ClO}^-$ . [16]

ORP is defined as the ease with which a substrate/medium loses or gains electrons and is measured in millivolts (mV) using an ORP meter. Measurement of ORP can be either positive or negative, and positive ORP indicates that a substance is an

oxidizing agent, higher ORP refers to more oxidizing it is. NaOCl as a common sanitizer usually has a ORP as 650-750mV. [17] EW has positive and higher ORP usually range from 600 to 1200mV, which is an important factor affect the antimicrobial efficacy of EW.

Early studies on the sanitizing application of EW were mostly focused on AEW, however, since the last decade, other variants of EW, have gained increasing popularity. Based on their pH values and chlorine concentration, EW could be classified into the following subgroups.

#### Acidic Electrolyzed Water (AEW)/Strongly Acidic Electrolyzed Water (StAEW):

AEW/StAEW is produced from the cathode of a two compartments electrolysis cell. AEW is characterized with a low pH value (pH 2-5) and high ORP value (>1100mV). [10], [18] In past years, its high sanitation efficacy is recognized by the food industry and started to be applied on food products. [19]

#### Slightly Acidic Electrolyzed Water (SAEW)/Weakly Acidic Electrolyzed Water (SAEW):

SAEW is characterized with a pH of 5 to 7 and ORP of 600-1000mV. [20] Unlike AEW, SAEW is produced by electrolysis of a mixture of HCl and tap water in an electrolytic cell with anode and cathode and no separating membrane. [21] Due to its relative high pH and less harmful to the environment, it gained much more researcher's interest in recent years.

#### Neutral Electrolyzed Water (NEW)

NEW is usually produced by electrolyzing NaCl with tap water, or diluting AEW with alkaline to retrieve a pH of 7.0 to 8.0 and ORP of 600 to 800 mV. Its neutral pH may pose less negative effect on human health and environment hence it receives many interest in recent years. [6]

#### Low concentration Electrolyzed Water. (ACC < 10 ppm, LcEW)

LcEW is often produced by electrolyzing a diluted NaCl solution with water in an EW generator that has no separate membrane. It has a pH of 6.0-6.5, OPR as 500-520mV and ACC less than 10 ppm. Its low concentration of chlorine and relative neutral pH may cause less erosion and negative effect on environment. [14], [22]

### III. ANTIMICROBIAL EFFECTS OF ELECTROLYZED WATER

The antimicrobial effects of EW have been proven with numerous reports. Here we briefly review some of the representative studies that focuses on microorganism that are of the highest importance from food safety concerns.

#### A. *Bacillus cereus*

As a toxin-producing bacterium, *B. cereus* produces enterotoxin, emetic toxin, and high resistant spores, which may contaminate food products and cause sickness in humans. It is estimated that there are 63,400 foodborne illness caused by *B. cereus* annually in the United States each year. [23]-[25] Sodium hypochlorite, hydrogen peroxide and peracetic acid are commonly used to kill

microorganism in food, however, spores are multi-stress resistant and conventional disinfecting treatments are less effective in killing spores compared to vegetative bacteria (Table I Entity 20). [26]

Hence, it is urgent for a novel disinfectant that is effective, nontoxic and environmentally safe to inhibit *B. cereus*. Multiple studies have suggested that EW is able to serve as a disinfectant for *B. cereus* contaminated food products, and their results were summarized in Table I. Zhang and co-workers conducted a study on the effect of EW on inactivating *B. cereus* in suspension and on carriers, it was showed that nondetectable level of *B. cereus* spores could be found in suspension after 2 min of exposure to both AEW and SAEW containing 120 mg/L ACC, and both types of EW water with ACC of 60 mg/L and 6 min treatment achieved a reduction of *B. cereus* spores to nondetectable level (Table I). [26] Biofilm formed by *B. cereus* is another challenge to food industry because biofilm is difficult to disperse in food plants due to its Extracellular Polymetric Substance (EPS). A study from Oh and co-workers applied AEW, SAEW and AIEW with varying pH and ORP values to the mature biofilms formed by *B. cereus* strains, ATCC 14579 and Korean Collection for Type Cultures (KCTC) 13153 on stainless steel and plastic slide coupon. SAEW (ACC, 25 ± 1.31mg/L; pH, 5.71 ± 0.16 and ORP, 818 to 855mV) was able to reduce *B. cereus* ATCC 14579 biofilms on

plastic slide coupon to non-detectable level within 0.5 min (Table I). [27] It was also reported that AEW (pH, 2.73; ACC, 79.6mg/L; ORP, 746mV) application to *B. cereus* biofilm on stainless steel surface achieved a 4.6 log CFU/cm<sup>2</sup> reduction, which is more effective compared to other chemically modified water (DI water, acetic acid solution, HCl solution, NaOCl solution). [28] Inhibitory effects of LcEW and other sanitizers against *B. cereus* on oyster mushroom were also investigated in a study conducted by Ding and co-workers. LcEW and StAEW showed strong inhibitory effects against *B. cereus*, which LcEW application on the oyster mushroom eliminated 2.03 log CFU/cm<sup>2</sup> of *B. cereus* at room temperature after 3 min immersion treatment and application of StAEW reached 1.96 log CFU/cm<sup>2</sup> reduction in the same setting. [26] Raw vegetables also pose a huge risk to human health because of contamination of *B. cereus*, a study was conducted on the inhibitory effect of 100 ppm Chlorine water, 100 ppm EW and 200 ppm EW for raw vegetables (cherry tomatoes, spring onions, Chinese chives and chicory), and after 3 types sanitizing treatments, microbial reduction effects showed 0.56-1.23 log for *B. cereus* and pose no significant differences in taste and flavor between with and without sanitizing treatments (Table I). [29]-[32]

TABLE I. ANTIBACTERIAL EFFECTS OF EW AGAINST B. CEREUS

Entity	EW				Treatment				Microbe concentration			Ref.
	Type	ACC/ppm	ORP/mV	pH	Condition	Temp	Time/min	Method	Before /log	After /log	Reduction /log	
1	AEW	120	1118-1188	2.62-2.90	suspension	r.t.	2	cover	7.57	<1	6.57	[26]
2	SAEW	120	920-956	5.81-5.99	suspension	r.t.	2	cover	7.57	<1	6.57	[26]
3	AEW	60	1118-1188	2.62-2.90	suspension	r.t.	2	cover	7.57	3.95	3.62	[26]
4	SAEW	60	920-956	5.81-5.99	suspension	r.t.	2	cover	7.57	4.22	3.35	[26]
5	SAEW	25	818-855	5.71	plastic slides	r.t.	0.5	dipping		N.D.		[27]
6	AEW	79.6	1177	2.73	stainless steel	r.t.	15	rinsing	4.66	N.D.	>4.0	[28]
7	LcEW	5	500-520	6.2	oyster mushroom	r.t.	3	immersion	7.18-7.55	5.28	1.9-2.27	[26]
8	SAEW	50	1100-1120	2.54	oyster mushroom	r.t.	3	immersion	7.18-7.55	5.35	1.83-2.2	[26]

N.D. = not detectable; r.t.= room temperature

### B. *Listeria monocytogenes*

*Listeria monocytogenes* is a Gram positive, facultative anaerobic and non-spore forming pathogen. *L. monocytogenes* presents in soil, damp and cool areas, animals, raw milk, and meat. *L. monocytogenes* is also associated with Ready-To-Eat foods because of its resistance to drying and freezing. Due to its characteristics of forming biofilm and survival ability in cold temperature, it caused about 1,600 listeriosis cases in the United States, and 260 people among them die each

year. (<https://www.cdc.gov/listeria/index.html>) Due to its' persistence in processing plants, it can cause big issue in food industries because it can be continually re-introduced into the plant environment and eventually lead to contamination of food contact surfaces and RTE products.

However, increasing recognition of EW pose a new hope for the food industry and increasing studies are conducted to investigate the inhibitory effect of *L. monocytogenes* by the application of EW.

In a study conducted by Ovissipour *et al.*, showed that Strong Acidic Electrolyzed Water (StAEW, 20mg/mL chlorine) and Weak Acidic Electrolyzed Water (WAEW, 10mg/mL) were able to completely inactivate (8.1 logs CFU/mL reduction) *L. monocytogenes* at concentrations of 107 CFU/mL or higher within 2 min in both selective and non-selective medias (Table II). In addition, AIEW with pH of 10.5-11.8 and ORP of -715mV to -890mV also showed the antimicrobial effect on *L. monocytogenes* by a reduction of 1-3 log/CFU in suspension. [32]

Application of AEW on food has been considered in various studies. Al-Holy studied the bactericidal efficacy of AEW (pH 2.30, ACC 38 ppm) on *L. monocytogenes* inoculated on raw trout skins, chicken legs and beef surface. In that study, the AEW resulted in approximately 1.2, 1.1 and 1.3 logs of *L. monocytogenes* reduction in trout, chicken and beef after 10 min treatment (Table II). [33] *L. monocytogene* is well adapted to harsh environmental conditions since they are halotolerant (10-20% salt), low-temperature-tolerant (less than 1°C), facultative anaerobic and highly acidic tolerant, hence it is a major concern to RTE foods producers. There was a study of the effect of EW on cold-smoked Atlantic salmon (*Salmo salar*). AEW (pH 2.7, ORP 1150 mV and ACC 60 ppm) was applied on *L. monocytogenes*-contaminated smoked salmon under different temperature (20, 30 and 40°C) at different times (2, 6 and 10 min). Treatment of AEW on smoked salmon at the temperature of 40°C for 10 min resulted in the largest reduction (2.85 log CFU/g) of *L. monocytogenes* while reduction of 1.41 to 2.85 log CFU/g were achieved under other time and temperature settings. [31] The presence of *L. monocytogenes* in food processing plants pose a huge risk to human health because *L. monocytogenes* generate biofilm that survive on the stainless steel and other surfaces. Hence, NEW was also investigated to observe the effect of inhibiting lux-tagged *L. monocytogenes* EGDe biofilms adhered to stainless steel. About 8 log CFU/mL free cells of *L. monocytogenes* were reduced to non-detectable levels after 1 min of

exposure to NEW (9 or 12mg/L of Total Available Chlorine (TAC), pH 6.5-7.5, ORP 800-1100mV). NEW with a 70mg/L TAC also exhibited the bactericidal effect of completely inhibiting 5 days old *L. monocytogenes* biofilm after 3 min of contact time on stainless steel. [33], [34] When comparing to conventional sanitizer such as NaClO (ACC 62mg/L), NEW (63mg/L) resulted in the same bactericidal effect to *L. monocytogenes* of both sanitizers with reducing it by 6 log CFU/ 50cm<sup>2</sup> after 1 min on stainless steel at 23°C. [24] NEW also showed bactericidally to *L. monocytogenes* on the surface of tomatoes by Deza and others. Two types of NEW that have pH of 8.2 ± 0.09 or 8.09 ± 0.05, ORP as 808 ± 7.5 or 760 ± 11 and ACC as 450 ± 11 or 92.10 ± 10mg/L were able to reduce *L. monocytogenes* from 7.51 log CFU/mL to nondetectable level in pure culture at the temperature of 23°C. In addition, washing tomatoes with diluted NEW (1:5 dilution with DI water) for 1 min also resulted 4.74 log CFU/cm<sup>2</sup> of *L. monocytogenes*, which doubled the quantity (2.65 ± 0.84 log CFU/cm) compared to DI water application. Also, application of NEW to tomatoes did not induce any sensory disadvantage. [30] Rahman and others applied low LcEW (pH, 6.3; ORP, 520mV; ACC, 5mg/L) to treat microorganism on spinach and LcEW significantly reduced the population of *L. monocytogenes* by 2.80 log CFU/g in 3 min of treatment at the r.t. of 23°C. It is also found that in comparison to other sanitizers such as SAEW (pH, 2.54; ORP, 1130mV; ACC, 50mg/L) and NaOCl, aqueous ozone and 1% citric acid, LcEW had stronger antimicrobial effects than NaOCl, aqueous ozone and 1% citric acid, and LcEW and SAEW did not differ significantly in reducing *L. monocytogenes* population on spinach. [22] In 2011, Rahman, Ding and others used the similar LcEW to investigate the bactericidal effect on oyster mushroom and found that application of LcEW to oyster mushroom resulted in greater sanitation effect (1.85 log CFU/g reduction) comparing to NaOCl, aqueous ozone and 1% citric acid and did not differ to SAEW significantly. [26]

TABLE II. ANTIBACTERIAL EFFECTS OF EW AGAINST *L. MONOCYTOGENES*

ENTITY	EW				TREATMENT				MICROBE CONCENTRATION			REF.
	TYPE	ACC /PPM	ORP /MV	pH	CONDITION	TEMP. /°C	TIME /MIN	METHOD	BEFORE /LOG	AFTER /LOG	REDUCTION /LOG	
1	LCEW	5	520	6.3	SPINACH LEAF	23	3	DIPPING	6.96	4.16	2.80	[22]
2	StEW	50	1130	2.54	SPINACH LEAF	23	3	DIPPING	6.96	4.26	2.70	[22]
3	NEW	450	808	8.2	PURE CULTURE	23	5	RINSING	7.51	<1	>6	[30]
4	NEW	92.10	760	8.09	PURE CULTURE	23	5	RINSING	7.51	<1	>6	[30]
5	NEW	92.10	760	8.09	TOMATO SURFACE	23	0.5	RINSING	5.34	0.73	4.66	[30]
6	NEW	92.10	760	8.09	TOMATO SURFACE	23	1	RINSING	5.34	0.54	4.74	[30]
7	AEW	60	1150	2.7	SALMON FILLET	20	6	IMMERSION	5.77	4.22	1.55	[31]
8	AEW	60	1150	2.7	SALMON FILLET	20	10	IMMERSION	5.77	4.19	1.58	[31]
9	AEW	60	1150	2.7	SALMON FILLET	40	6	IMMERSION	5.77	3.41	2.36	[31]
10	AEW	60	1150	2.7	SALMON FILLET	40	10	IMMERSION	5.77	2.92	2.92	[31]
11	ALEW	ND	-715 TO -890	10.5-11.8	SELECTIVE MEDIA	20	6	WASHING	8.10	6.10	2.0	[32]
12	LCEW	5	500-520	6.2	OYSTER MUSHROOM	23	3	IMMERSION	7.18-7.55	5.39	2.16	[26]
13	StAEW	50	1100-1120	2.54	OYSTER MUSHROOM	23	3	IMMERSION	7.18-7.55	5.47	2.08	[26]
14	NEW	63	774.0	7.79	STAINLESS STEEL	23	1	RINSING	6.95-8.84	N.D.	>6	[24]
15	NEW	63	774.0	7.79	GLASS SURFACE	23	1	RINSING	6.95-8.84	N.D.	>6	[24]

16	NEW	63	774.	7.79	PURE CULTURE	23	5	RINSING	7.5	N.D.	>7	[24]
17	AEW	38	/	2.30	FISH	22	10	SOAKING	/	/	1.2	[33]
18	AEW	38	/	2.30	CHICKEN	22	10	SOAKING	/	/	1.1	[33]
19	AEW	38	/	2.30	BEEF	22	10	SOAKING	/	/	1.3	[33]
	LCEW	5	500	6.30	LETTUCE LEAF	35	1	DIPPING	6.97	3.21	3.76	[14]
	StAEW	50	110	2.60	LETTUCE LEAF	35	1	DIPPING	6.971	3.29	3.68	[14]

N.D = NOT DETECTABLE

TABLE III. ANTIBACTERIAL EFFECTS OF EW AGAINST E. COLI

ENTITY	MICROBE	EW				TREATMENT				MICROBE CONCENTRATION			REF.
		TYPE	ACC /PPM	ORP /MV	pH	CONDITION	TEMP /°C	TIME /MIN	METHOD	BEFORE /LOG	AFTER /LOG	REDUCTION /LOG	
1	E. COLI O157:H7	SAEW	30	915-984	5.61-6.32	SUSPENSION	R.T.	0.5	MIXING	/	/	9	[34]
2		AEW	20	1043-1144	2.71-2.89	SUSPENSION	R.T.	0.5	MIXING	/	/	9	[34]
3		NEW	432	816	8.03	PURE CULTURE	23	5	RINSING	7.45	<1	>7	[30]
4		NEW	86.40	771	8.15	PURE CULTURE	23	5	RINSING	7.45	<1	>7	[30]
5		NEW	86.40	771	8.15	TOMATO SURFACE	23	0.5	RINSING	5.46	1.11	4.35	[30]
6		NEW	86.40	771	8.15	TOMATO SURFACE	23	1	RINSING	5.46	0.54	4.92	[30]
7		SAEW	2	812.5	6.53	SUSPENSION	25	3	IMMERSION	8.0	ND	8	[35]
8		SAEW	120	857-889	6.23-6.48	MUNG BEAN SPROUTS	20	15	SOAKING	6.22	1.71	4.51	[36]
9		SAWE	80	817-879	6.13-6.38	MUNG BEAN SEEDS	20	15	SOAKING	5.79	1.55	4.24	[36]
		NEW	37	818	7.64	PURE CULTURE	R.T.	5	MIXING	8.46	<1.62	>8	[37]
		NEW	74	818	7.64	PORK SKIN	R.T.	2	IMMERSION	5.17	2.97	2.2	[37]
		NEW	74	818	7.64	PORK SKIN	R.T.	10	IMMERSION	5.17	2.58	2.59	[37]
		NEW	74	818	7.64	PORK CHOP	R.T.	2	IMMERSION	5.16	4.84	0.32	[37]
		NEW	74	818	7.64	PORK CHOP	R.T.	10	IMMERSION	5.16	4.87	0.29	[37]
		LCEW	5	500	6.30	LETTUCE LEAF	35	1	DIPPING	8.02	5.53	2.49	[14]
		StAEW	50	1100	2.60	LETTUCE LEAF	35	1	DIPPING	8.02	5.52	2.50	[14]
	E. COLI (NBRC3301)	SAEW	22.1	931	5.6	CHINESE CELERY	20	5	DIPPING	8.18	5.44	2.74	[21]
		SAEW	22.1	931	5.6	LETTUCE	20	5	DIPPING	8.31	5.47	2.84	[21]
		SAEW	22.1	931	5.6	DAIKON SPROUTS	20	5	DIPPING	8.57	5.72	2.85	[21]
		SAEW	23.7	940	5.6	SUSPENSION	20	1	MIXING	9.4	3.79	5.61	[38]
		SAEW	23.7	940	5.6	SUSPENSION	20	2	MIXING	9.4	3.13	6.27	[38]
		SAEW	23.7	940	5.6	SUSPENSION	20	3	MIXING	9.4	2.7	6.69	[38]
		StAEW	50.3	1139.4	2.6	SUSPENSION	20	1	MIXING	10-11	4.39	6.02	[38]
	E. COLI CECT 405	NEW	63	774.0	7.79	STAINLESS STEEL	23	1	RINSING	6.95-8.84	0.27	>6	[24]
		NEW	63	774.0	7.79	GLASS SURFACE	23	1	RINSING	6.95-8.84	0.24	>6	[24]
		NEW	63	774.0	7.79	PURE CULTURE	23	5	RINSING	7.5	N.D.	>7	[24]

N.D. = NOT DETECTABLE; R.T.= ROOM TEMPERATURE

### C. *Escherichia coli* O157:H7

As a shiga toxin producing pathogen, consumption of low dosage of *E. coli* O157:H7 is able to induce hemorrhagic colitis, hemolytic uremic syndrome, thrombotic thrombocytopenic purpura and death incident in human body. [35]-[37] *E. coli* O157:H7 can be transmitted among cattles through fecal to oral route and through food vehicles such as ground beef, raw milk, apple cider and raw vegetables. Traditionally, food industry applies interventions such as hot water and organic acids (lactic acid, acetic acid) on carcass to eliminate the contamination. Recently, multiple studies were conducted to use EW as a novel sanitizer, and the results were summarized in Table III. Zhang and others treated viable assay of *E. coli* O157:H7 with SAEW, AEW and NaOCl with different ACC. SAEW (ACC, 10-50mg/L; pH, 5.61-6.32; ORP, 915-984mV) and AEW (ACC, 5-40mg/L; pH, 2.71-2.89; ORP 1043-1144mV) showed better bactericidal effect on *E. coli* O157:H7 than NaOCl (ACC, 20-80mg/L; pH, 7.87-8.64; ORP, 816-841mV). 50, 40 and 70mg/L ACC of SAEW, AEW and NaClO are required to completely inactivate *E. coli* O157:H7. [34] In another study, SAEW with different ACC, treatment times and temperatures were applied to observe the bactericidal effect on *E. coli* O157:H7. Application of SAEW that has a pH 6.0-6.5, at ACC of 2mg/L and ORP as  $812.5 \pm 8.0$ mV on *E. coli* O157:H7 resulted in 100% inactivation at the temperature of 25°C in a 3 min treatment. [35]. Al-Holy studied the bactericidal efficacy of AEW that have pH 2.30, free chlorine 38 ppm on the *E. coli* O157:H7 inoculated on raw trout skins, chicken legs and beef surface. In this study, the AEW exhibited a significant bactericidal on *E. coli* O157:H7-contaminated raw fish, beef and chicken by reducing the population about 1.0 and 1.5 log CFU/g after 5 and 10 min treatment time. [33] Application of AEW has strong and aggressive effect on foodborne pathogens, hence scientist investigated in NEW due to its neutral pH that does not aggressively cause corrosion of processing plant and irritation of hands. Deza and others studied the bactericidal effect of two types of NEW to *E. coli* O157:H7. NEW and NEW-diluted that have pH of  $8.03 \pm 0.23$  or  $8.15 \pm 0.20$ , ORP as  $816 \pm 9.0$  or  $771 \pm 7.0$  and ACC as  $432 \pm 5.1$  or  $86.40 \pm 4.1$ mg/L were able to reduce *E. coli* O157:H7 from 7.45 log CFU/mL to nondetectable level in pure culture at the temperature of 23°C. In addition, washing tomatoes with NEW-diluted for 60 s also resulted in 4.92 log CFU/cm<sup>2</sup> reduction of *E. coli* O157:H7, which doubled the quantity (2.65 log CFU/cm<sup>2</sup>) compared to DI water application. Also, application of NEW to tomatoes did not induce sensory difference. [30] These studies indicate LAEW, NEW and AEW can be potential sanitizer to inactivate *E. coli* O157:H7.

### D. *Salmonella enteritidis*

*S. enteritidis* usually cause self-limiting gastrointestinal disease in human. It is estimated that *Salmonella* spp causes about 1.2 million illness, 23,000 hospitalization and 450 deaths in the U.S every year (<https://www.cdc.gov/salmonella/index.html>) However,

the toughness to detect *S. enteritidis* in back yard poultry products limits the intervention of the contamination. Conventionally, prevention methods of *S. enteritidis* are adequate cooking of foods and good personal hygiene. Hence, it is urgent for the food industry to testify if a novel sanitizer can inactivate *S. enteritidis* in food products. Table IV summarizes the effects of EW on *S. enteritidis* inactivation.

Shimamura and others investigate the bactericidal effect of combination of AIEW and StAEW in their sterilization of chicken breasts in 4 or 25°C. Combination of AIEW (pH 11.5) and StAEW (pH, 2.5; ORP,  $975 \pm 15$ mV; ACC,  $30 \pm 2.0$ mg/L) at 4°C reduced the population of *S. enteritidis* in chicken by 3.46 log CFU/g. At the r.t. (25°C), combination of AIEW (pH 11.5) and StAEW (pH, 2.5; ORP, 960mV; ACC,  $14 \pm 1.5$ mg/L) reduced the population of *S. enteritidis* in chicken sample by 3.25 log CFU/g. It is al shown that application of the combination of AIEW and StAEW did not have significant difference in sensory evaluations from the control samples. [18]. Zhang and others applied SAEW with a pH of 5.98-6.38, ORP of 817.5-889.0mV and ACCs of 20, 40, and 60mg/L to inactivate *S. enteritidis* on mung beans. Application of SAEW than has an ACC of 80mg/L for 15 min resulted in largest reduction of *S. enteritidis*. (4.19 log CFU/g reduction) [36] Issa-Zacharia also studied the effect of SAEW as a sanitizer to RTE vegetables (daikon spouts, lettuce and Chinese celery) on *Salmonella* spp. In that study, the antimicrobial effect of SAEW (pH, 5.6; ORP, 931 mV; ACC, 22.1mg/L) and NaClO (pH, 9.6; ORP, 646 mV; ACC, 107mg/L) was investigated. Application of both chlorinate sanitizers significantly affect the population of *Salmonella* spp. by reducing approximately 2.9 log CFU/g in 3 Ready-To-Eat vegetables. [21] Han and others studied the antimicrobial efficacy of NEW on pork products and pure cultures, in addition, they observe the formation of Viable but Nonculturable Cells (VBNC) after treatments. Application of NEW (pH,  $7.64 \pm 0.07$ ; ORP,  $818 \pm 5.57$ mV; ACC 37 ppm) for *S. enteritidis* resulted in complete inactivation of *S. enteritidis* and no VBNC cells were detected. Inoculated pork chops and skin samples were treated with 100% NEW (ACC 74 ppm) for 2 or 10 mins. *S. enteritidis* on pork skin experienced a significant population reduction, with a 2.22 log CFU/cm<sup>2</sup> reduction observed after 2 mins treatment and 2.37 log CFU/cm<sup>2</sup> reduction observed after 10 mins treatment. However, there was no significant reduction of *S. enteritidis* population was observed in the application of 100% NEW in porkchops. This study also proved that sensory or quality changes were not observed in the application of NEW on pork skin or pork chops. [37] NEW was also studied by Deza and others to investigate the antimicrobial effect on washing tomatoes. NEW and NEW-diluted that have pH of 7.99 or 8.19, ORP as 795 or 745 and ACC as 465 or 93.00mg/L were able to reduce *S. enteritidis* from 7.70 log CFU/mL to nondetectable level in pure culture at the temperature of 23°C. In addition, washing tomatoes with NEW-diluted for 1 min also resulted 4.30 log CFU/cm<sup>2</sup> of *S. enteritidis* which

doubled the quantity (2.20 log CFU/cm) compared to DI water application. Also, application of NEW to tomatoes did not induce sensory difference. [30]

#### E. *Staphylococcus aureus*

*S. aureus* infections are often seen in communities and hospital settings, however, it is remained challenge to prevent it due to its multi-drug resistance. [38], [39] In

the past decades, several studies have been carried out to investigate the antimicrobial effects of EW against *S. aureus*, and some of the most representative results were shown in Table V. It is clear that all EW showed high potency on inhibiting the growth of *S. aureus* in suspension and sanitizing fresh produce contaminated by *S. aureus*.

TABLE IV. ANTIBACTERIAL EFFECTS OF EW AGAINST SALMONELLA SPP

ENTITY	MICROORGANISM	EW				TREATMENT				MICROBE CONCENTRATION			REF.
		TYPE	ACC /PPM	ORP /MV	PH	CONDITION	TEMP /°C	TIME /MIN	METHOD	BEFORE /LOG	AFTER /LOG	REDUCTION /LOG	
	SALMONELLA SPP. (NBRC 13245)	SAEW	22.1	931	5.6	CHINESE CELERY	20	5	DIPPING	8.35	5.48	2.87	[21]
		SAEW	22.1	931	5.6	LETTUCE	20	5	DIPPING	8.60	5.69	2.91	[21]
		SAEW	22.1	931	5.6	DAIKON SPROUTS	20	5	DIPPING	8.58	5.67	2.91	[21]
		SAEW	21.4	940	5.7	SUSPENSION	20	1	MIXING	9.4	3.81	5.60	[38]
		SAEW	21.4	940	5.7	SUSPENSION	20	2	MIXING	9.4	3.24	6.17	[38]
		SAEW	21.4	940	5.7	SUSPENSION	20	3	MIXING	9.4	2.61	6.80	[38]
		STAEW	50.6	1140	2.6	SUSPENSION	20	1	MIXING	10-11	4.61	6.12	[38]
	S. ENTERITIDIS	NEW	37	818	7.64	PURE CULTURE	R.T.	5	MIXING	8.41	<1.62	>8	[37]
		NEW	74	818	7.64	PORK SKIN	R.T.	2	IMMERSION	3.97	1.75	2.22	[37]
		NEW	74	818	7.64	PORK SKIN	R.T.	10	IMMERSION	3.97	1.6	2.37	[37]
		NEW	74	818	7.64	PORK CHOP	R.T.	2	IMMERSION	4.02	4.02	/	[37]
		NEW	74	818	7.64	PORK CHOP	R.T.	10	IMMERSION	4.02	4.02	/	[37]
		NEW	465	795	7.99	PURE CULTURE	23	5	RINSING	7.70	<1	<7	[30]
		NEW	93.00	745	8.19	PURE CULTURE	23	5	RINSING	7.70	<1	<7	[30]
		NEW	93.00	745	8.19	TOMATO SURFACE	23	0.5	RINSING	5.16	1.49	3.67	[30]
		NEW	93.00	745	8.19	TOMATO SURFACE	23	1	RINSING	5.16	0.86	4.30	[30]
		SAEW	120	847-893	6.03-6.29	MUNG BEAN SPROUTS	20	5	SOAKING	5.67	1.44	4.23	[36]
		SAWE	80	837-889	5.98-6.27	MUNG BEAN	20	15	SOAKING	5.49	1.30	4.19	[36]
	SALMONELLA TYPHYMURIUM	LCEW	5	500	6.30	LETTUCE LEAF	35	1	DIPPING	7.03	3.39	3.64	[14]
		STAEW	50	1100	2.60	LETTUCE LEAF	35	1	DIPPING	7.03	3.5	3.53	[14]

N.D. = NOT DETECTABLE; R.T.= ROOM TEMPERATURE

TABLE V. ANTIMICROBIAL EFFECT OF EW AGAINST YEAST AND MOLD

ENTITY	EW				TREATMENT				MICROBE CONCENTRATION			REF.
	TYPE	ACC /PPM	ORP /MV	PH	CONDITION	TEMP /°C	TIME /MIN	METHOD	BEFORE /LOG	AFTER /LOG	REDUCTION /LOG	
1	LCEW	5	500-520	6.2	OYSTER MUSHROOM	23	3	IMMERSION	4.73	3.65	1.08	[26]
2	STAEW	50	1100-1120	2.54	OYSTER MUSHROOM	23	3	IMMERSION	4.73	3.71	1.02	[26]
3	LCEW	5	520	6.30.2	SPINACH LEAF	23	3	DIPPING	2.41		1.64	[22]
4	SAEW	21-22	500-600	5.2-5.5	CHINESE CABBAGE	23	3	IMMERSION	/	/	1.40	[40]
5	SAEW	21-22	500-600	5.2-5.5	LETTUCE	23	3	IMMERSION	/	/	1.41	[40]
6	SAEW	21-22	500-600	5.2-5.5	SESAME LEAF	23	3	IMMERSION	/	/	1.24	[40]
7	SAEW	21-22	500-600	5.2-5.5	SPINACH	23	3	IMMERSION	/	/	1.19	[40]

### F. *Pseudomonas aeruginosa*

As a Gram-negative, rod-shaped pathogen, *P. aeruginosa* grows well at the temperature from 25 to 37°C hence it is ubiquitous in nature. *P. aeruginosa* is a common nosocomial contaminant and it causes between 10% to 20% of infections in most hospitals. Serious infections including malignant external otitis, endophthalmitis, endocarditis, meningitis, pneumonia and septicemia can be caused by infection of *P. aeruginosa*. [40], [41] Hence, it is important to discover inhibitor of *P. aeruginosa* to reduce the infection rate. Surface and equipment are commonly being contaminated with pathogens because of their moisture and residues of foods. In one study, NEW that has pH of 7.79, ORP of 774.0mV and ACC as 63 ppm was applied to, pure culture stainless steel and glass to inhibit *P. aeruginosa*. 1 min treatment of NEW resulted in 7.5 log CFU/mL and 6 log CFU 50 /mL reduction of *P. aeruginosa* on pure culture, stainless steel and glass in r.t. (23±2°C). Comparing to traditional disinfectant in household and food industry, the bactericidal efficacy of NEW on glass and stainless steel surface is similar to that of NaClO in that study. [24] RTE food products, such as fresh cut vegetables pose tremendous risk to human health because they are normally served uncooked and act as carrier for foodborne pathogens (Table VI). Another study suggested that NEW is able to reduce *P. aeruginosa* in fresh-cut food product. [42], [43]

### G. Yeast and Mold-fungi

Few studies have showed that application of EW can effectively inactivate yeast and mold and reduce the load in food products such as RTE vegetables and pork. 3 mins treatment on Chinese lettuce, sesame leaf and spinach with SAEW that has pH of 5.2-5.5, ORP as 500-600 mV and ACC of 21-22 mg/L could successfully result in 1.40, 1.41, 1.24 and 1.19 log CFU/g reduction of yeast and mold at r.t (Table VII). [40] In another study, Oh and others treated spinach that is contaminated with yeast and

mold with LcEW (pH, 6.3±0.2; ORP, 520 ± 20mV; ACC, 5 ± 0.1mg/L) and StAEW (pH, 2.54 ± 0.3; ORP, 2.54 ± 0.3; ACC 50 ± 2.2mg/L). 3 mins application of LcEW resulted in 1.64 log CFU/g reduction of yeast and mold in spinach at r.t. and StAEW resulted in similar reduction. Application of LcEW and StAEW were also able to reduce 1.35 log CFU/g of yeast and mold on oyster mushroom. [22], [26] Other than vegetables, the bactericidal efficacy of LcEW was investigated against yeast and mold on pork. Meat samples were sprayed for 2 min with LcEW (pH, 2.15 ± 0.02; ORP:1159.32 ± 29.6mV; ACC: 16.60 ±0.24mg/L) and it is observed a 2.68 log CFU/cm<sup>2</sup> of yeast and mold. In addition, the application of LcEW did not significantly affect color of meat products. [44]

### H. Human Norovirus

Of the 15 leading U.S. foodborne pathogens, Norovirus sickens about 61% of population. The only host for it is believed to be humans and fecal-oral route is the primary transmission because they do not reproduce or grow in foods. Foods that are easily contaminated with Norovirus include water, fresh produce (such as vegetables, fruits), shellfish and prepared food. [45] Due to its resistance toward heating to 60°C, drying (for week) and can survive in soil and water for months, it is tough to eliminate Human Norovirus except for disinfectants such as bleach and inhibit cross contamination. Hence, novel EW may serve as a disinfectant to it. 30 mins of NEW (ACC, 250 ± 2.0 ppm; ORP, 974.7 ± 2.7mV and pH, 6.2;) application on stainless steel resulted in 5.0 log reduction of Human Norovirus, which may serve as antiviral disinfectant to eliminate cross contamination of Human Norovirus. However, the presence of soil load greatly affects the antiviral efficacy of NEW to only produce less than 0.3 log reduction (Table VIII). [41] There are insufficient studies conducted on the antiviral efficacy of EW and further investigation is needed.

TABLE VI. ANTIMICROBIAL EFFECTS OF EW AGAINST PSEUDOMONAS SPP

ENTITY	EW				TREATMENT				MICROBE CONCENTRATION			REF.
	TYPE	ACC(PPM/MG/L)	ORP /MV	pH	CONDITION	TEMP /°C	TIME /MIN	METHOD	BEFORE /LOG	AFTER /LOG	REDUCTION /LOG	
1	NEW	63	774.0	7.79	STAINLESS STEEL	23	1	RINSING	6.95-8.84	0.56	6.39	[24]
2	NEW	63	774.0	7.79	GLASS SURFACE	23	1	RINSING	6.95-8.84	0	>6	[24]
3	NEW	63	774.0	7.79	PURE CULTURE	23	5	RINSING	7.5	N.D.	>7	[24]

N.D. = NOT DETECTABLE;

TABLE VII. ANTIMICROBIAL EFFECT OF EW AGAINST HUMAN NOROVIRUS

ENTITY	EW				TREATMENT				MICROBE CONCENTRATION			REF.
	TYPE	ACC /PPM	ORP /MV	pH	CONDITION	TEMP /°C	TIME /MIN	METHOD	BEFORE /LOG	AFTER /LOG	REDUCTION /LOG	
8	NEW	250	974.7	6.2	SUSPENSION WITHOUT SOIL LOAD	R.T.	1	PIPPETED	/	/	5.4	[41]
	NEW	250	974.7	6.2	STAINLESS STEEL WITHOUT SOILD LOAD	R.T.	30	PIPPETED	/	/	5.0	[41]
	NEW	250	974.7	6.2	STAINLESS STEEL WITH SOILD LOAD	R.T.	30	PIPPETED	/	/	<0.3	[41]

TABLE VIII. ANTIBACTERIAL EFFECTS OF EW AGAINST STAPHYLOCOCCUS AUREUS

ENTITY	EW				TREATMENT				MICROBE CONCENTRATION			REF.
	TYPE	ACC /PPM	ORP /MV	PH	CONDITION	TEMP /°C	TIME /MIN	METHOD	BEFORE /LOG	AFTER /LOG	REDUCTION /LOG	
1	LCEW	5	500	6.30	LETTUCE LEAF	35	1	DIPPING	7.07	3.08	3.99	[14]
2	StAEW	50	1100	2.60	LETTUCE LEAF	35	1	DIPPING	7.07	3.31	3.76	[14]
3	NEW	63	774	7.79	STAINLESS STEEL	23	1	RINSING	6.95-8.84	0.27	>6	[24]
4	NEW	63	774	7.79	GLASS SURFACE	23	1	RINSING	6.95-8.84	0.24	>6	[24]
5	NEW	63	774	7.79	PURE CULTURE	23	5	RINSING	7.5	ND	>7	[24]
6	SAEW	23.7	940	5.6	SUSPENSION	20	1	MIXING	9.3	3.95	5.33	[38]
7	SAEW	23.7	940	5.6	SUSPENSION	20	2	MIXING	9.3	3.34	5.94	[38]
8	SAEW	23.7	940	5.6	SUSPENSION	20	3	MIXING	9.3	2.92	6.36	[38]
9	StAEW	50.3	1139.4	2.6	SUSPENSION	20	1	MIXING	10-11	4.25	5.93	[38]
10	SAEW	21.1	948	5.8	PURE CULTURE	20	0.5	MIXING	/	/	2.75	[39]
11	SAEW	21.2	948	5.8	PURE CULTURE	20	1	MIXING	/	/	4.81	[39]
12	SAEW	21.2	948	5.8	PURE CULTURE	20	1.5	MIXING	/	/	5.32	[39]
13	StAEW	45.3	1140	2.6	PURE CULTURE	20	0.5	MIXING	/	/	3.33	[39]
14	StAEW	45.3	1140	2.6	PURE CULTURE	20	1	MIXING	/	/	5.94	[39]
15 16	StAEW	45.3	1140	2.6	PURE CULTURE	20	1.5	MIXING	/	/	6.78	[39]

#### IV. ANTIMICROBIAL MECHANISM

##### A. Cell Membrane Damage

Large body of studies have revealed that cell membrane can be damaged by the application of EW. Outer cell membrane of *E. coli* O78 and *S. aureus* 26003 were thinned and destroyed after treatment with StAEW and SAEW, and inoculum of these two bacteria become lighter in color and deformed. [19] It is found that the disinfection mechanism of SAEW is to disrupt the permeability of cell membrane and cytoplasmic ultrastructure in *S. aureus* cells based on the measurement of intracellular potassium leakage, TTC-dehydrogenase relative activity and the observed changes of ultrastructure. [46]-[48] Through measurement of crystal violet uptake, the deterioration of cell membrane permeability was seen in *E. coli* and *Enterococcus faecalis* after treatment of EW. [49], [50] In comparison to other disinfectant, EW poses more severe membrane damage to pathogen due to the presence of active species or oxidizing agents such as free radicals and hydrogen peroxide. The whole cell death is characterized by cell membrane damage and further macromolecular leakage. [51] It is demonstrated that cell membrane is the primary target of EW that produced HClO and OCl<sup>-</sup>. [23], [52]

##### B. Ion and Protein Leakage

Studies have shown that the application of EW could alter cell membrane permeability and cause iron and protein leakage. In a study investigating the mechanism of EW as a disinfectant to *Aspergillus flavus*, it is found that the permeability of K<sup>+</sup> and Mg<sup>2+</sup> were significantly increased and the leakage ultimately increased after the treatment of NEW (pH, 6.0; ORP: 845mV; ACC: 114.3mg/L) and AEW (pH: 2.2; ORP, 1146mV; ACC: 161.6mg/L) due to the damage on the functional unit of *A. flavus*. [53]-[57] Application of SAEW (ACC of 33mg/L, pH 6.4, ORP of 834.9mV) also induce K<sup>+</sup> leakage of

108.34% after the treatment to *S. aureus* in pure culture, and K<sup>+</sup> leakage is significantly higher than that of NaClO with ACC of 27mg/L and 0.1% HCl. [48] Other studies also suggest the ion leakage were seen after treatment of EW on foodborne pathogen and serves as a disinfectant mechanism. [49], [57] It is suspected that potassium leakage resulted from the disruption of membrane protein. [23]

Proteins are found on the cell membrane or intracellular which plays a significant role in pathogens because they are required for function, structure and regulation and excess protein leakage may result in cell death. An investigation of EW, NaOCl, and ClO<sub>2</sub> disinfectant efficacy on *Enterococcus faecalis* indicates that protein leakage increased after EW treatment and EW treatment for 5 and 10 min resulted in higher protein leakage than that of NaOCl. [51] Both SAEW and StAEW resulted in intracellular protein leakage of *E. coli*, *S. aureus* and *Bacillus subtilis*. [19] Plenty of objects have observed the significant protein leakage after EW treatment on foodborne pathogens due to the altered permeability of cell membrane. [58]-[64]

##### C. Inactivation of Enzymes

Enzymes are vital for the survival of life hence deterioration of enzyme can be fatal to pathogens. SAEW (pH 6.1, ORP 863.5mV, ACC 30mg/L) was studied as a treatment of *E. coli* and *S. aureus* and it targeted the intracellular esterase activity and inactivate the intracellular TTC (2,3,5-triphenyl tetrazolium chloride) dehydrogenase. [20], [23] It is suspected that chlorine compound is able to form N-Cl bonds with enzymes, which result in enzyme inactivation. [65] In addition, it is suggested that high ORP in EW inactivate enzymes. HClO as an oxidative substance can oxidize dehydrogenase and other enzymes in the respiratory system of bacteria. [48] Inactivation of TTC suggests the cells may have problems with respiration and anabolism when chlorinated EW is applied on the pathogen. [51]

#### D. Intracellular Reactive Oxygen Species and Involvement of Free Radical

Studies have demonstrated that electrolysis of water produce ROS, which was reported being a disinfectant mechanism of EW to pathogen. [57], [66]-[68] Yang and others reported that the application of EW on *Listeria innocua* resulted in increased generation of ROS, which serves as a disinfectant mechanism. [63] In addition, Jeong in 2006 demonstrated that ROS serve as an additional disinfectants contributed to anti-microorganism, similar to chlorine compounds. [69] However, recent study showed that intracellular ROS level of *E. coli* and *S. aureus* were not increased after the treatment of SAEW, and the result indicated that intracellular oxidation by SAEW mainly related to the chemical oxidants rather than ROS accumulation in cells. [23] Hence the mechanism of ROS still needs to be furthered studied.

#### E. DNA Damage and Leakage

Due to the deterioration of cellular membrane integrity, intracellular DNA may be damaged or being leaked to the extracellular environment. Treatments of EW treatment on *E. faecalis* resulted in significantly DNA leakage [51] Leakage of DNA was also observed after the treatment of EW on *Candida albicans*, *E. coli*, and *S. aureus*. [59], [64] Treatment of SAEW on Gram-negative *E. coli* and Gram-positive *S. aureus* induced DNA damage due to altered permeability of cell membrane. [23] More DNA damage was observed in *E. faecalis* after 30 s and 1 min treatments of EW than that of NaOCl. [70]-[72] Both treatment of StAEW and SAEW resulted in DNA damage and SAEW caused more severe DNA damage in *E. coli*, *S. aureus*, and *B. subtilis*. [19] It is concluded that the free radicals in EW contributed to damage DNA strands and caused base alterations. [73] DNA damage and leakage are detrimental to cells hence it may be deduced as a potential disinfection mechanism of EW.

#### F. Disruption of Extracellular Polymeric Substances

Extracellular Polymeric Substances (EPS) are secreted by the accumulation of planktonic cells in biofilm to protect bacteria being damaged and disruption of EPS can be detrimental to microorganisms. [73] Treatment of AEW on *Pseudomonas fluorescens* biofilm resulted in dramatic decrease of protein and carbohydrate concentration of EPS and may serves as a mechanism contributing to cell distortions and death. [62] However, there were no sufficient amount of study to determine this mechanism hence more studies should be conducted of EPS disruption in pathogen.

### V. FACTORS AFFECTING THE ANTIMICROBIAL EFFICACY OF EW

#### A. Storage Conditions

Storage conditions may be a factor that influence antimicrobial efficacy of EW and there is insufficient study dedicated on this factor. One study shows that in open storage chlorine were completely lost after 30 h when agitated and after 100 h when non agitated. ORP of

EW also decreased during open storage, however, pH of EW remained stable and lighting did not influence chlorine and ORP lost. The chlorine lost was mostly due to evaporation of chlorine gas. In closed condition, EW lost 60% of chlorine after 1400 h when light diffuses and 40% of chlorine in dark condition. In addition, ORP did not differ significantly and only decreased about 15 mV, and pH remain stable through storage. Diffused light had larger effect on chlorine loss. Mechanism of chlorine lost might be due to the chlorine self-decomposition under closed condition. [74] Conclusively, storage condition of EW can affect chlorine lose in EW and given that antimicrobial efficacy is highly affected by chlorine compound, storage condition may affect antimicrobial efficacy.

#### B. Electrolysis Condition

Rahman and others demonstrated that increasing current up to 1.45-1.47 A during electrolysis can produce more stable LcEW and minimize the available chlorine loss due to increased exposure time. It is observed that increased reduction of *E. coli O157:H7* and *L. monocytogenes* can be obtained by LcEW with increased current during electrolysis. Increasing current also induce the values of pH and ORP, which may affect the antimicrobial efficacy of EW. [75]

Electrolysis times may serve as a factor that affect the antimicrobial efficacy of EW as well and increased electrolysis time resulted in high ACC, lower pH, larger ORP and eventually larger reduction of *E. coli O157:H7*. [75]

It is demonstrated that EW water was able to cause log reduction of *E. coli O157:H7* and *L. monocytogenes* in wide range of pH (2.6-7) if sufficient ACC present. [76] Also, concentration of free chlorine increases with the increase electrolysis time and concentration of NaCl in electrolysis. [77]-[80]

#### C. Exposure Time

It has been shown that the increasing EW treatment time resulted in significant increased log reduction of *B. subtilis* and *B. cereus* spores. [26] Increased exposure time to EW also increase the log reduction of *Campylobacter jejuni*. [32] It is suggested by another study that increasing exposure time for EW treatment can increase antimicrobial efficacy on *L. monocytogenes*-contaminated cold-smoked salmon. [31] Large body of studies have showed that increased exposure time of EW to foodborne pathogen can enhance log reduction, which indicates the antimicrobial efficacy of EW. [38], [39], [41], [62]

#### D. Water Hardness

Water hardness was investigated as a factor affect not only the properties of EW but also the antimicrobial efficacy. Increased water hardness from 0 to 50mg/L resulted in increased log reduction of *E. coli O157: H7* from 5.8 to 6.4 log CFU/mL due to 40% increased free chlorine level as water hardness increases. However, elevated water hardness to 200mg/L did not affect antimicrobial efficacy of EW. [81]

### E. Organic Matters

Small amount of studies investigated the antimicrobial efficacy of EW when organic matters involved. Sporicidal efficacy of EW on *Bacillus* spores was reduced due to the presence of organic matter. [26] Presence of organic matter in AEW that treated on contaminated lettuce and spinach leave decreased the disinfectant efficacy of AEW on *E. coli* O157:H7, *Salmonella* Typhimurium and *L. monocytogenes*. [80] Another study on the antimicrobial efficacy of NEW on pork skin and pork chop also suggested that the organic matter on Pork chop negatively affect the antimicrobial efficacy of NEW for *S. enteritidis*, *E. coli* O157:H7 and *Yersinia enterocolitica*. [37] Presence of sufficient amount of chlorine compounds can penetrate cell membrane. [81]-[83] Organic compounds can react with available chlorine and lead to lower antimicrobial efficacy of EW. [84]

### F. Treatment Temperature

It is investigated that increased temperature (4 to 40°C) for EW treatment can significantly increase the antimicrobial efficacy in inhibiting *E. coli* O157:H7, *L. monocytogenes*, *S. typhimurium* and *B. cereus*. [26] Similar results also observed by other researchers. [31], [37]

## VI. EFFECTS OF ELECTROLYZED WATER TREATMENT ON FOOD QUALITY AND NUTRITION

### A. Fruits and Vegetables

Adverse effects of disinfectant may lower customer satisfaction on food products hence the effect of EW on food products should be investigated to ensure the applicability of EW on food products or food plants to minimize foodborne infection. SAEW treatment on RTE vegetables and sprouts can effectively reduce *E. coli* and *Salmonella* spp. and reduce the amount of chlorine used and has less adverse effects that hypochlorite solution pose. In addition, SAEW has neutral pH, which may minimized corrosion of surface and affect human health. [21] Application of EW on green onions and tomatoes to inhibit *E. coli* O157:H7, *Salmonella typhimurium* and *L. monocytogenes* also did not affect the appearance, taste or color of the vegetables. [78] Sensory evaluation was conducted on tomatoes that were sanitized with NEW, and there were no significant difference in taste, appearance or smell was detected by panelist. [30] Another study suggest that NEW that contains lower ACC (60 ppm) can be applied on lettuce for disinfectant without interfering the quality of lettuce. [85] Yet in a study that investigate the antimicrobial efficacy of SAEW on mung bean showed that longer treatment time (15 min) can result lowered germination percentage after treatment. [36] However, future studies to focus on the investigation on the quality and nutrition value of various vegetables after application of EW.

### B. Seafood and Fish

Application of AEW to control foodborne pathogen did not affect show apparent deterioration in the quality

of shellfish. [32] It is suggested that the AEW treatment on smoked-salmon did not alter texture, color and sensory perception of the food product. [31] Application of AEW ice on shrimp has demonstrated the potential ability in minimizing pH changes of shrimp flesh and significantly retarding the change of color, and it posed no adverse effect on the firmness of shrimp while had a capability to inhibit foodborne pathogen in shrimp. [85] However, there is insufficient studies investigate the effect of EW on seafood and fish quality hence more investigation should conducted.

### C. Meat

Color deterioration was observed after the application of LcEW on pork due to the changes in total hemoglobin, however, the color change was not significant and meat structure was not affected. [44], [61] Combination treatment of AIEW and StAEW on chicken and beef liver showed no significant changes in pH, lipid oxidation, color, amino-acid content, texture and sensory properties when comparing to the untreated products. [18]

## VII. CONCERNS AND OUTLOOK

Large body of studies have been conducted on the efficacy to EW as a disinfectant and EW showed to be equally or even greater antimicrobial efficacy than other chlorine sanitizers. [1], [2] LcEW pose a large potential to be used as an effective sanitizer against foodborne pathogen, and due to its neutral pH, low concentration of chlorine that cause less negative on environment compared to other sanitizers. [24]-[26] However, EW remains novel to customer and food industry because insufficient amount of investigation conducted on the safety of using EW on food products and production plant. In addition, studies should be conducted on the hurdle method of EW combined with other technique to more effectively inhibit foodborne pathogen and spoilage microorganisms. Study of food quality after treatment of EW are limited and further study should be conducted for actual application in food industry to testify the efficacy in various food products and plants.

## CONFLICT OF INTEREST

The author declares no conflict of interest.

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