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Recent advances on research of electrolyzed water and its applications

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As one of the most promising sterilization agents for microbial control in food industry in recent decades, electrolyzed water (EW) can be produced from diluted NaCl solution, and exhibits strong broad-spectrum bactericidal efficiency due to the synergistic effect of available chlorine concentrations, pH and oxidation reduction potential. To date, numerous studies have demonstrated the antimicrobial activity of EW against various kinds of microorganisms both *in vitro* and *in vivo*. However, the exact antimicrobial mechanisms of EW have not been determined at present, limiting its widespread application. In this review, we provide an overview of latest production equipment of EW, and briefly summarize the current advances of germicidal factors and antimicrobial mechanisms of electrolyzed water on different states of microorganisms. In addition, studies about hurdle enhancement of EW combined with other technologies are also discussed, providing guidelines for improving food safety and food quality both in conventional and organic food industry.

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Introduction

Foodborne disease has become a critical health problem around the world. According to Centre for Disease Control and Prevention (CDC) statistics, more than 250 foodborne diseases have been identified so far, resulting in 48 million people get sick each year in the United States. More specifically, around 128 000 are hospitalized and 3000 die annually, causing suffering to patients and bringing financial burden to society. In 2016 annual report released by CDC, most foodborne illnesses were caused by norovirus, followed by *Salmonella* and Shiga toxin-producing *Escherichia coli* successively [1]. Therefore, cleaning and sanitization is one of the most critical steps to ensure food safety under the monitoring of Hazard Analysis and Critical Control Point (HACCP) system during food processing.

At present, numerous commercial sanitizers, such as chlorine compounds, peroxide mixtures, quaternary ammonium compounds and ozone, have been used as disinfection techniques throughout the food supply chain. However, some of these techniques could not be completely acceptable when applied to food products, due to some disadvantages such as potential toxicity to human being or environment, chemical residues, limited inactivation effectiveness and adverse effects on food quality. Therefore, development of effective and safe sanitizers in food industry has become an ongoing subject of interest [2,3].

Electrolyzed water (EW), produced by electrolyzing dilute NaCl (sometimes KCl or $MgCl_2$) solution in an electrolysis chamber, has become one of the most promising sterilization agents for hygiene control in food industry in recent two decades, due to its effective antimicrobial activity and low-cost running expense [4,5•]. According to the pH value of final solution, EW can be classified into several types, including acidic EW (pH 2.2–2.7), weakly acidic EW (pH 2.7–5.0), slightly acidic EW (pH 5.0–6.5), neutral EW (pH 6.5–7.5) and alkaline EW (pH 11.0–13.8).

To date, numerous published papers and books have demonstrated the antimicrobial activity of different kinds of EW against various kinds of microorganisms both *in vitro* and *in vivo*, as well as in various physiological states, providing great realistic guiding significance to the fundamentals and applications of EW technology in food sanitation [6]. Although the exact antimicrobial mechanisms of EW have not been determined at present, great progress has been achieved in recent years in the knowledge of EW's disinfection efficacy on different food matrices, as well as its effect on food's physicochemical properties throughout the post-harvest storage. Therefore, this review introduces recent advances on the fundamentals of EW, unravelling key contributing factors to its antimicrobial capacity. In addition, recent studies on the latest applications of EW in different food sectors are also summarized, demonstrating current situation and development trend of EW to a full-fledge commercial scale.

Types of EW-producing systems and factors influencing the antimicrobial activity of EW

In general, EW is generated by electrolysis of dilute NaCl solution in an electrolytic chamber, which is usually divided into two types: two-cell chamber containing a diaphragm between the anode and cathode electrodes for acidic and alkaline EW production, and single-cell chamber without the separating membrane for neutral and slightly acidic EW production [7]. Chlorine compounds (HOCl, Cl_2, ^{-}OCl) produced through a series of reactions in the electrolysis system are main factors responsible for the bactericidal ability of EW. However, they tend to react with organic matters (e.g. amino acids, proteins) existing on food matrices, thereby weakening the sterilizing capacity of EW and limiting its wide applications in food industry [8]. The good news is that at the moment, some new electrolyzed water generators have been developed to overcome such limitations. For example, a new circulating electrolyzed water (CEW) device was introduced in recent years through modifying the traditional EW generator, by controlling the switches on and off to get CEW after repetitive electrolysis, which can increase the stability of EW with higher available chlorine concentration obtained in comparison to slightly acidic EW (Figure 1b) [9••]. Moreover, even in diluted form, CEW also showed greater efficacy in reducing microbes on pork and lettuce, without compromising their physicochemical characteristics [10]. On the other hand, considering current commercial EW-generating equipments are extremely large and inconvenient for use in households and diminutive food industries, Zhang, Yang and Chan [11] developed a portable flow-through, neutral EW-producing unit recently, and found that the neutral EW generated through redirecting cathode products back to the anode chamber had stronger germicidal effect than its counterpart produced by redirecting anode solution back to the cathode chamber, which can be served as a promising sanitizing unit for consumers. The detailed schematic illustration of the unit and the related reaction pathways in the anode chamber are shown in Figure 1a.

The basic properties of EW include available chlorine concentration (ACC), pH and oxidation-reduction potential (ORP), which are regarded as three main factors directly influencing EW's sanitizing efficacy. Numerous studies have found out the interaction effects among them, such as pH value could alter the formation of chlorine species and the ORP value could decrease markedly when the pH increased, which had mutual effects during the process of sterilization [12]. On the other hand, free radicals (such as hydroxyl radicals ($^{\bullet}OH$)) are also considered as germicidal components of EW, although some previous studies' results were conflicting [13,14]. It's worth mentioning that all electrolytic cells producing $^{\bullet}OH$ reported before were batch units, but recently, a portable sanitizing unit mentioned above could produce neutral EW containing $^{\bullet}OH$ and $O_2^{\bullet -}$ continuously. The $^{\bullet}OH$ could react with Cl^- as an alternative way to produce $Cl^{\bullet}$, as well as involving in several reactions to yield H_2O_2, O_2 and H_2O, while the $O_2^{\bullet -}$ could react with H_2O to produce H_2O_2 or with O_2 to form O_3 (Figure 1a). One modification of this portable EW generation unit by redirecting the cathode products containing hydroxide ions is that it could promote the production of $^{\bullet}OH$ and $O_2^{\bullet -}$, which is different from existing common EW generation systems-based mainly on chlorine sanitization. Therefore, the formation of chlorine in this modified EW could be produced from two perspectives, including the direct electro-catalysis of Cl^- and the indirect oxidation of Cl^- mediated by $^{\bullet}OH$ and other free radicals. The increased production of free radicals could contribute to a greater antimicrobial efficacy of EW by enhancing the presence of $^{\bullet}OH$ and $O_2^{\bullet -}$ [11].

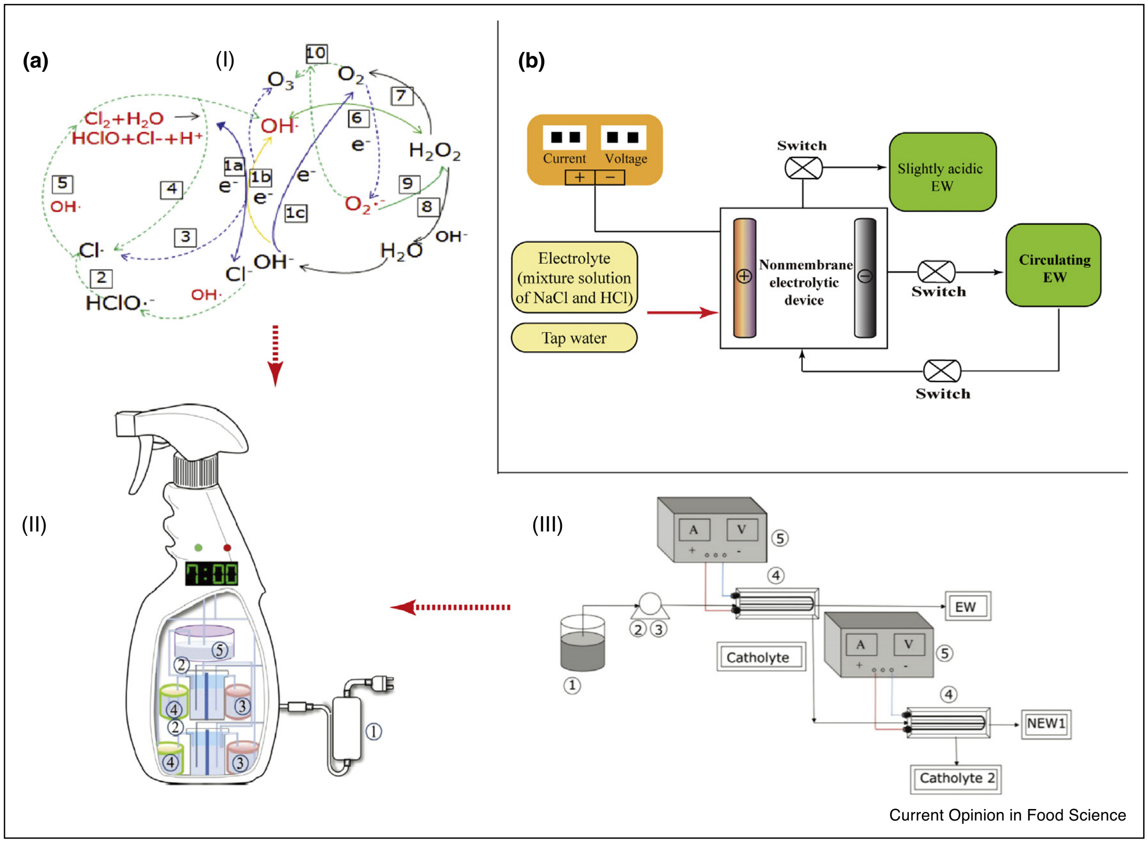
The germicidal mechanisms of EW on microorganisms

At present, the germicidal mechanisms of EW have not been completely elucidated, but a model explaining the germicidal mechanisms of EW roughly has been developed and shown in Figure 2a. In brief, EW exhibited its germicidal property by attacking multiple cellular targets (cytoderm, outer membrane and intracellular components). Firstly, the morphology of cell surfaces was changed from smooth, consecutive and bright into rough, shrunken, and even lysed after being treated with EW [15]. Meanwhile, the bacterial protective barriers (cell wall and membrane) were attacked and destructed by chlorine species, which could increase membrane permeability and the leakage of intracellular compounds (K^+, proteins and DNA) [16].

After EW diffusing through membrane, HOCl and produced reactive oxygen species (ROS) would induce a complex series of changes of intracellular metabolites. Microbial metabolomics analysis has become an increasingly powerful approach recently in the area of 'omics' research, providing new biomarkers for microbiology studies, which is helpful to understand the actual physiological state of the cell [17]. However, limited research has been done on this subject, let alone EW's effects on the bacterial metabolite profile changes. Liu *et al.* [18,19••] used nuclear magnetic resonance (NMR) spectroscopy coupled with multivariate statistical analysis to investigate the global metabolic responses of Gram-negative and Gram-positive bacteria to EW oxidative stresses (e.g. hydroxyl radicals [$^{\bullet}OH$]), respectively, and the overview of metabolic alterations is shown in Figure 2b1 and b2.

HOCl and produced hydroxyl radicals(such as O^-, Cl^-, and OH^-) significantly disordered normal cellular functions and cellular ultrastructures through different degrees, including: (1) metabolic level: changing the metabolic state, including the inhibition of nucleotide

Figure 1



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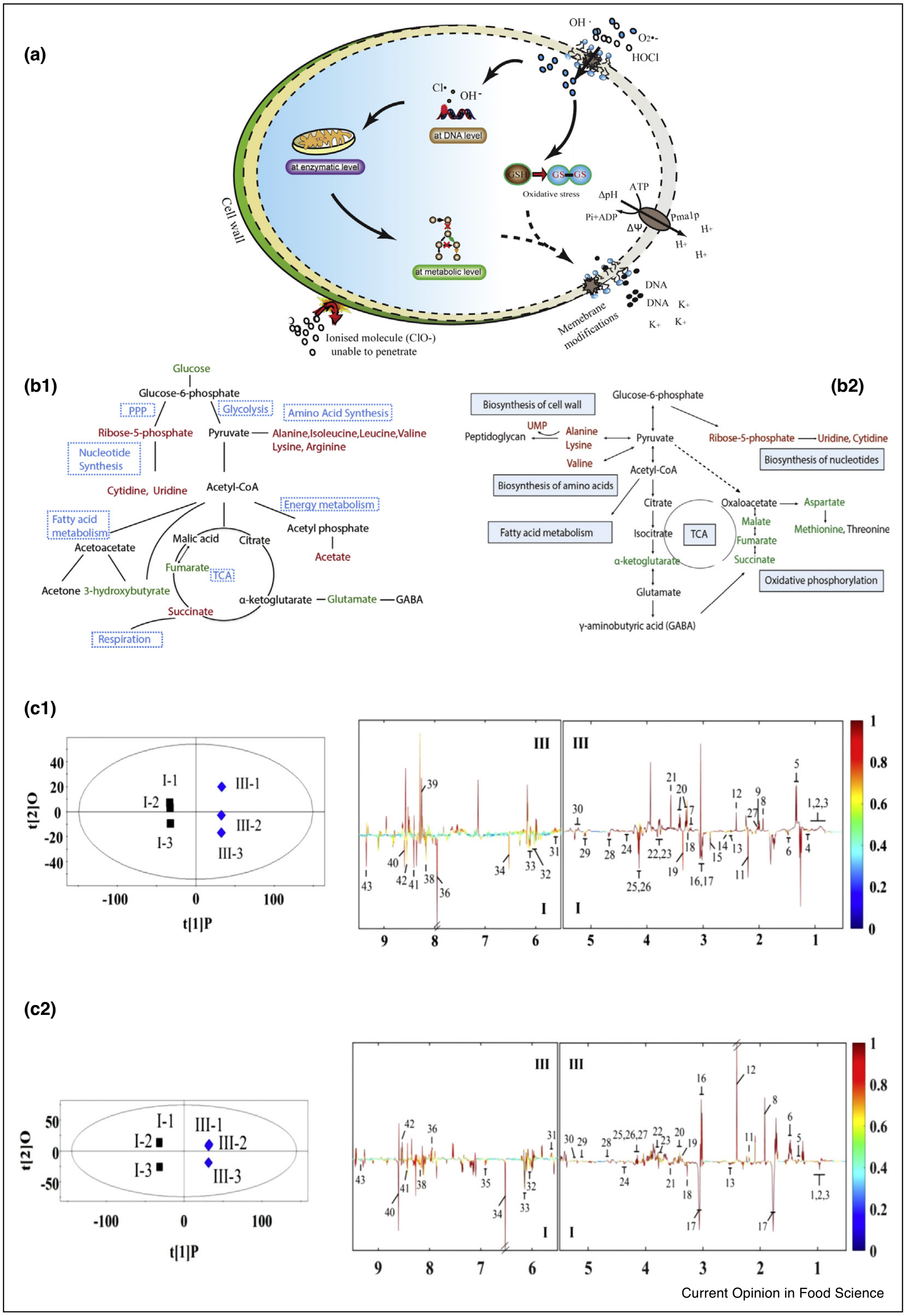
Overview of electrolyzed water generator systems developed recently. **(a)** A portable flowthrough neutral EW producing unit [11] (**I**: Schematic reactions in the anode chamber for producing neutral EW. Substances colored in red: the detected products and their intermediates; Dotted arrows: the reactions have not been verified definitely in this study; Green arrows: pathways involved in radical chain reaction mechanisms; Blue arrows: pathways involved in catalytic reaction mechanisms; Black arrows: the general chemical reactions; Orange solid arrows: the verified reaction steps in this study. **II**: A designed model for portable neutral EW sanitizing generator. 1, power supply; 2, electrolytic tank; 3, tank containing EW; 4, tank containing catholyte solution; 5, tank containing neutral EW. **III**: Schematic diagram of the portable flowthrough neutral EW producing system, in which neutral EW is generated by redirecting the cathode products back to the anode chamber. 1, electrolyte solution; 2, pump; 3, controller; 4, electrolysis chamber; 5, power supply). **(b)** Schematic diagram of a circulating EW generation unit with a nonmembrane electrolytic cell [9••]. Sub-graphs (a) are reproduced with permission from John Wiley and Sons, and sub-graph (b) is adapted from Ref. [9••].

and amino acid biosynthesis, the suppression of energy-associated metabolism (glycolysis and ATP replenishment), and the enhancement of fatty acid metabolism [18]; (2) enzymatic level: decreasing the activities of several key enzymes, or enhancing glutamate decarboxylase (GAD) system and γ-aminobutyric acid (GABA) shunt to elevate the levels of α-ketoglutarate and succinate [19••]; (3) intracellular microenvironment: decreasing intracellular ATP level and pH value, and enhancing the release of ROS to induce cell necrosis and apoptosis [20].

In addition to lethal effect, EW could induce microorganisms to transfer into other physiological states, such as sublethal injury and viable but non-culturable (VBNC) state, in which microorganisms could wait for resuscitation when the conditions become suitable to grow [21,22]. However, most studies only focused on the count and proportion of microorganisms induced by EW to enter these states, the molecular mechanisms underlying the transfer should be further investigated in depth.

Furthermore, different states of microorganisms are supposed to exhibit different responses and sensitivities to external treatments. Recently, more researchers have paid attention to evaluate the antimicrobial mechanisms of EW against microorganisms in different states. As for the biofilm-forming bacteria, EW treatment firstly triggered the disruption of extracellular polymeric substances through deforming the carbohydrate C–O–C bond and aromatic rings in tyrosine and phenylalanine, and then rapidly eradicated biofilms and decreased population of biofilm cells [23]. Moreover, for the air-dried bacteria attached on stainless steel coupons, EW induced a series

Figure 2



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(a) A summarized model explaining the germicidal mechanism of EW from multiple attacking targets [51]. **(b)** Overview of the significant metabolite changes after EW treatment. Metabolites plotted in green and red indicate an increased and decreased level in EW stressed cells respectively compared to untreated cells. Metabolites in black were not verified in this study. **b1** is for *E. coli* (Gram-negative representative) and **b2** is for *L. innouca* (Gram-positive representative) [18,19••]. **(c)** Orthogonal projection to latent structure discriminant analysis score plots (left) and coefficient-coded loading plots (right) in *E. coli* cells upon EW treatment. **c1** is for planktonic *E. coli* and **c2** is for air-dried *E. coli*, respectively. I: deionised of metabolomic variations in the air-dried cells, which was different from those in planktonic counterparts (Figure 2c1 and c2). For example, the levels of a wide range of metabolites in air-dried *E. coli* extracts, such as acetate, taurine, alanine and glutamate were increased after EW treatment, while the contents of most metabolites in planktonic *E. coli* were decreased, showing different germicidal mechanisms of EW on different states of microorganisms [24••].

Hurdle enhancement of EW with other treatments in organic food industry

During the production and practice process of EW, the levels of NaCl, current values, electrolysis time, water property (e.g. water temperature and hardness), water flow rate, electrode materials, storage environment, agitation and organic compounds could all effectively influence the physicochemical characteristics of EW, and then further affect its sanitization efficacy [25]. Therefore, EW in combination with other technologies has become a universal hurdle enhancement method in food decontamination processing, showing synergistic effects on inactivating targeted microorganisms and extending food product's shelf life, while maintaining food quality and nutritional value.

Considering the hurdle enhancement of EW treatments has developed more than 15 years, numerous studies of EW combined with other technologies have been reported, as shown in Table 1. It is worth mentioning that the organic food market has developed rapidly over recent years worldwide, however, organic food has brought great safety challenge to public, due to the strict regulations prohibiting the use of chemosynthetic pesticides and its vulnerability to be contaminated by pathogens [26]. The good news is that recently there's increasing study and focus on organic-compatible sanitizing approach to guarantee organic food security, and EW treatment alone or in combination with others is one of the hot points of the research.

Organic acids

Generally, most of organic acids (such as lactic acid, levulinic acid, citric acid, fumaric acid) are recognized as safe to meet the strict regulations of organic food, and exhibit strong bactericidal effects on various pathogens, in which the disinfection efficacy depends on the pKa with non-dissociated form, as well as donated hydrogen ions in an aqueous system [27]. Recently, the combined treatment of EW and organic acids was developed and showed synergetic effects on inhibiting pathogens growth, and then significantly improve microbial safety for various food products. For example, combining 3% levulinic acid with low concentration acidic EW (free available chlorine [FAC]: 4 mg/L) could be a potential sanitizing method in organic food industry, meeting organic operation standards and significantly decreasing the population of *E. coli* and *Listeria innocua* with 3.5–4.0 log CFU/g, without changing the quality of organic lettuce during storage [28]. However, in another study, the combined treatment of EW (FAC: 4 mg/L) and citric acid (0.6%) showed limited antimicrobial effect on both organic and conventional fresh-cut lettuce, indicating the parameters associated with different combined treatments should be optimized to obtain better effectiveness and suitability of the process [4].

Heat processing

Thermal processing could be applied to decrease the population of microorganisms and inactivate enzymes to prolong the shelf life of products, but easily alters food quality such as taste loss and nutrient degradation. At present, the combination of EW and mild-thermal processing (also known as mildly heated EW) has been applied as an effective control measure to maintain the quality and safety for fresh-cut and ready-to-eat organic produce. For example, after treated by the combination of EW (FAC: 4 mg/L) and mild heat (50°C), fresh organic broccoli could still maintain its quality and nutritional value while the amount of natural microbiota or inoculated pathogens on it were decreased significantly. Moreover, according to the morphology observation from atomic force microscopy, this combined method could induce pectin chains to form a self-assemble network, contributing to the improved firmness of organic broccoli [29]. Similarly, the synergistic inactivation effects of EW (FAC: 4 mg/L) and short-time heat treatment (60, 70 and 80°C) on organic carrot were fitted by Weibull model, describing the inactivation kinetics of *E. coli* O157:H7 and *S.* Typhimurium in detail during 3-min treatment. By taking into account the shelf life evaluation as well, treatment of EW at 70°C for 1 min was the best sanitizing method for organic carrots in this study, improving food safety and maintaining good quality during storage [30•].

Ultrasound

While most studies about sanitizing practice were focused on food itself, EW as a novel food contact surface sanitizer should be paid more attention. As microbial colonization on food contact surfaces (e.g. plant processing equipment,

(Figure 2 Legend Continued) water treatment (control); III: EW treatment. Metabolites: 1: isoleucine; 2: leucine; 3: valine; 4: β-hydroxybutyrate; 5: lactate; 6: lysine; 7: arginine; 8: acetate; 9: acetamide; 11: β-aminoadipate; 12: succinate; 13: aspartate; 14: methylamine; 15: trimethylamine; 16: γ-aminobutyrate; 17: putrescine; 18: betaine; 19: methanol; 20: taurine; 21: glycine; 22: alanine; 23: glutamate; 24: uridine; 25: glyceric acid; 26: phosphorylcholine; 27: *N*-acetyl alanine; 28: β-glucose; 29: phosphoenolpyruvate; 30: α-glucose; 31: ribose-5-phosphate; 32: cytidine; 33: adenosine 2′-3′-cyclic phosphate; 34: fumarate; 35: tyrosine; 36: xanthine; 37: uridine 5′-monophosphate; 38: hypoxanthine; 39: nicotinate; 40: adenosine monophosphate; 41: formate; 42: inosine triphosphate; 43: nicotinamide adenine dinucleotide [24••]. Sub-graph (a) is adapted from Ref. [51]. Sub-graphs (b and c) are reproduced with permission from Elsevier.

Table 1

Combination of electrolyzed water and various preservation technologies to guarantee food quality and safety

Combined treatments	Food matrices	Microorganisms (reduction log CFU/g)	Other effects	Refs
AEW + ozone (200 mg/L)	Tangerine	Inhibiting the spore germination of fungus	Extending the storage life, controlling postharvest decay	[33]
AlEW + 1% citric acid at 50°C	Shredded carrots	*Listeria. monocytogenes* (3.97 log CFU/g)	Improving sensory quality, and prolonging shelf-life	[34]
NEW + nisin (6976 IU/per coupon)	Glass and stainless steel surfaces	*L.monocytogenes* (4.81 log CFU/cm^2)	–	[35]
NEW + ultrasound (20 kHz, 130 W and 210 W)	Lettuce	*E. coli* O157:H7 (4.4 log CFU/g) and *S*. Typhimurium DT 104 (4.3 log CFU/g)	Having no effect on food quality, and extending the shelf life	[36]
Low concentration EW + 3% calcium lactate	Fresh pork	*E. coli* O157:H7 and *L. monocytogenes* (3.0–3.2 log CFU/g)	Extending the shelf life	[37]
SAEW + 0.5% fumaric acid	Fresh beef	Total viable count (TVC) (>2.6 log CFU/g)	Prolonging the shelf life	[38]
AEW+ 2% chitosan	American shad (*Alosa sapidissima*)	Suppressing microbial growth	Inhibiting protein decomposition and lipid oxidation	[39]
SAEW + thermosonication (400 W/L, 40°C)	Fresh-cut Kale	*L. monocytogenes* (6 log CFU/g); *E. coli O157:H7* (3.32 log CFU/g); *L. monocytogenes* (3.11 log CFU/g)	Prolonging the shelf life	[40]
SAEW + 0.5% FA at 40°C	Fresh pork	*E. coli* O157:H7 (2.59 log CFU/g), *L. monocytogenes* (2.69 log CFU/g), *S. aureus* (2.38 log CFU/g), and *S*. Typhimurium (2.99 log CFU/g)	Prolonging the shelf life, and improving sensory quality (color, odor, and texture)	[41]
SAEW + ultrasound (40 kHz, 400 W/L) at 60°C	Fresh-Cut Bell Pepper	*L. monocytogenes* and *S. enterica serovar* (2.70 log CFU/g)	Having no effect on the color and hardness, and prolonging the shelf-life	[42]
AlEW + strong AEW	Fresh chicken breasts	*S. Enteritidis* NBRC 3313*, E. coli* ATCC 10798*, S. aureus* FDA209P*, and S. aureus* C-29 (>1.0 log CFU)/g)	–	[43]
SAEW + ultrasound (40 kHz, 400 W/L) + mild heat (40, 50, 60°C)	Fresh-cut bell pepper	*L.monocytogenes* (3.0 log CFU/g) and *S*. Typhimurium (3.0 log CFU/g)	–	[44]
SAEW + 0.2% CaO + 0.5% FA + ultrasonication (40 kHz, 400 W/L)	Apple	*E. coli* O157:H7 (4.28 log CFU/fruit) and *L. monocytogenes* (5.25 log CFU/fruit)	Maintaining the quality of fruits	[45]
AEW+ 0.5% carvacrol nanoemulsion	Fresh-cut vegetables	Aerobic mesophilic and psychrotropic bacteria counts (0.5 log CFU/g)	Prolonging the antimicrobial activity of AEW	[46]
Low concentration EW + mild heat (50°C)	Fresh organic broccoli	Effectively inhibiting *E. coli* O157:H7 and *L. monocytogenes*	Maintaining the antioxidant content, total phenolic levels and ferric reducing antioxidant power	[29]
AEW + ultraviolet light (254 nm) + ultrasound (45 kHz, 200 W)	Raw salmon fillets	Total viable count (TVC) (0.64 log CFU/g)	Having no effects on the texture and firmness of tissue	[47]
Low concentration NEW + ultrasound (37 kHz, 80 W)	Stainless steel coupon	*E. coli* ATCC 25922 (2.2 log CFU/coupon), *P. pastoris* GS115 (3.1 log CFU/coupon) and *A. pullulans* 2012 (1.0 log CFU/coupon)	–	[5•]
SAEW + high pressure processing (200 and 500 MPa for 20 min sequentially)	Mud snail (*Bullacta exarata*)	Total microbial counts and psychrotrophic bacteria (<1.0 log CFU/mL)	Having no effect on the essential amino acids	[48]
Low concentration AEW + ultrasound (40 kHz, 200 W)	Fresh-sliced button mushrooms	Controlling the population of total bacteria (TBC), yeast and mold	Prolonging the shelf life, and delaying surface browning	[49]
NEW + mild thermal processing (65°C)	Atlantic salmon fillets	*L. monocytogenes* (5.6 log10 CFU/g)	Having no effect on the protein structure	[50]
Low concentration AEW + 3% levulinic acid	Fresh organic lettuce	*E. coli* ATCC 25922 and *L. innocua* ATCC 33090 (3.5–4.0 log CFU/g)	–	[28]

Note: AEW, acidic electrolyzed water; AlEW, alkaline electrolyzed water; NEW, neutral electrolyzed water; SAEW, slightly acidic electrolyzed water; FA, fumaric acid.

household kitchen items) is a quite common phenomenon, sanitizing method that can remove bacteria or biofilms effectively from food contact surface is of great significance in ensuring food safety. Ultrasound has been widely used in industrial fields for a long time due to its antimicrobial effect, but its application as an antimicrobial agent in food processing is more recent [31]. Whereas ultrasound alone in most cases does not inactivate bacteria effectively, combination treatment of ultrasound with other technologies could achieve better sanitizing results. For example, combining ultrasound with EW (FAC: 4 mg/L) could cause the most significant reduction of *E. coli*, *Pichia pastoris* and *Aureobasidium pullulans* from stainless steel coupons, as the cells in suspension were killed by EW after being detached from coupons by ultrasound. Therefore, the combination of EW (FAC: 4 mg/L) and ultrasound could be developed as a short-time food contact surface sanitizing method for food industry, especially for organic food processing [5•].

These are some recent advances concerning the combination of EW and other technologies applied in organic food sanitization area. However, the combined processes should be further improved through the optimization of various operating parameters (e.g. concentration, pH and time), and then the more appropriate combinations with various preservation technologies could be developed to guarantee the safety, sensory quality and nutritional value of food.

Conclusions and future prospects

As an ecofriendly technology, electrolyzed water exhibits strong germicidal activity against various microorganisms ranging from bacteria to viruses. After approving by the United States Department of Agriculture (USDA) as generally recognized as safe, EW is gradually accepted around the globe, and then begins to be used as a sanitizer in the food industry, especially in organic food industry when its chlorine concentration fell to 4 mg/L (according to the regulations released by National Organic Program [NOP] of USDA, [52]). More importantly, with the development of portable electrolyzed water generators, EW has great possibility to be used for households in the next step. Although a model explaining the germicidal action of EW has been developed, indicating that the antimicrobial effect of electrolyzed water is determined by the capacity of chlorine species (such as HOCl and OCl^-) to a large extent, its germicidal mechanism has not been completely elucidated. Fortunately, metabolomics strategy gives a new insight into the antimicrobial mechanism of EW by analyzing microbials' global metabolic responses, with the aid of multivariate data analysis. Recently, to further improve the antibacterial activity of EW, the hurdle technology, combining two or more scaffold techniques, has been developed and shows synergistic effects in terms of decreasing microbial population, prolonging product's shelf life and maintaining product's quality.

In the future, to further improve the sanitizing efficiency of EW, the strategy will continue depending on a two-pronged approach that involves production and practice manipulations [32], including: 1) combining powerful tools (such as flow cytometer and electron microscopy), and molecular biology (including transcriptomics, proteomics, metabolomics and other omics) to provide more fundamental understandings on the sterilization mechanisms of EW related to multiple cellular targets, such as cell membrane, gene expression, metabolite levels, metabolic process, metabolic profiling, and cellular process; 2) integrating disinfection technologies and the disinfection mechanisms of EW to develop a hurdle method with synergistic effects for further improving the antimicrobial effect.

Conflict of interest statement

Nothing declared.

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- •• of outstanding interest

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