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DOI: <https://doi.org/10.4315/0362-028X-71.9.1934>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-4971>

Journal Article

Accepted Version

Originally published at:

Hricova, D; Stephan, Roger; Zweifel, C (2008). Electrolyzed water and its application in the food industry. *Journal of Food Protection*, 71(9):1934-1947.

DOI: <https://doi.org/10.4315/0362-028X-71.9.1934>

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## Review

### Electrolyzed Water and its Application in the Food Industry

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*Keywords:* Electrolyzed Water, Disinfectant, Processing Surfaces, Food Products

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**Abstract**

Electrolyzed water (EW) is gaining popularity as a sanitizer in the food industries of many countries. By electrolysis a dilute sodium chloride solution dissociates into acidic electrolyzed water (AEW; pH 2 to 3; oxidation-reduction potential (ORP) >1100 mV; active chlorine content 10-90 ppm), and basic electrolyzed water (BEW; pH 10 to 13; ORP -800 to -900 mV). By the use of AEW, vegetative cells of various bacteria in suspension were generally reduced by >6.0 log CFU/ml. However, influenced by factors such as surface type and the presence of organic matter, AEW is less effective on utensils/surfaces and food products. Reductions (log units) of bacteria obtained on surfaces/utensil and vegetables/fruits mainly ranged from about 2.0 to 6.0, and 1.0 to 3.5, respectively. Higher reductions were in particular obtained for tomatoes. For chicken carcasses, pork, and fish reductions ranged from about 0.8 to 3.0, 1.0 to 1.8, and 0.4 to 2.8, respectively. Considerable reductions yielded the use of AEW on eggs. On some food commodities, treatment with BEW followed by AEW showed stronger activity than treatment with AEW only. The EW technology deserves consideration in discussing possibilities for the industrial sanitizing of equipments and the decontamination of food products. Nevertheless, decontamination treatments for food products should always be seen as a part of an integral food safety system. Such treatments cannot replace strict adherence to good manufacturing and hygiene practices.

Cleaning and sanitizing are important elements of the hygiene measures conducted in a food processing plant. Typical sanitizers applied in the food industry include chlorine compounds, organic acids, trisodium phosphate, iodophors, and quaternary ammonium compounds (QAC). Chlorine compounds are often the most effective, although they may be more corrosive and irritating than alternatives such as iodine and QAC. Chemical substances are also used for decontamination purposes on certain food products. In the United States (US), decontamination treatments with antimicrobials have been authorized for carcasses, whereas such treatments are at present not permitted in the European Union. Some of these procedures have been found not to be acceptable due to chemical residues, high cost, limited effectiveness or discoloration of products.

Currently, the use of electrolyzed water (EW) is gaining popularity as a sanitizer in the food industry to reduce or eliminate bacterial populations on food products, food-processing surfaces, and non-food contact surfaces. In Japan, the Health, Labor and Welfare Ministry has officially approved EW as a food additive (110). Moreover, EW generator have also been approved for applications in the food industry by the US Environmental Protection Agency (EPA) (87). The purpose of this review is to give an overview of issues related to EW, its antimicrobial activity, and its application in the food industry (surfaces, process water, various food products).

## CONCEPT OF EW

**History.** The concept of EW has originally been developed in Russia, where it has been used for water decontamination, water regeneration, and disinfection in medical institutions (58, 59, 77, 78). Since the eighties, EW has also been used in Japan. One of the first applications of EW was the sterilization of medical instruments in hospitals (61, 98). Later on it has been utilized in various fields such as

agriculture or livestock management (4, 17, 99), but the use of EW was restricted by its short shelf life. With recent improvements in technology and the availability of better equipment, EW has gained interest as a disinfectant in the food industry.

**Generation.** EW is the product of the electrolysis of a dilute sodium chloride (NaCl) or KCl/MgCl<sub>2</sub> solution in an electrolysis cell, within which a diaphragm (septum or membrane) separates the anode and cathode. The basic principle of producing EW is shown in Fig. 1. The voltage between the electrodes is generally set at 9 to 10 volts (5). During electrolysis, NaCl dissolved in deionized water dissociates into negatively charged chlorine (Cl<sup>-</sup>) and positively charged sodium (Na<sup>+</sup>). At the same time, hydroxide (OH<sup>-</sup>) and hydrogen (H<sup>+</sup>) ions are formed. Negatively charged ions such as Cl<sup>-</sup> and OH<sup>-</sup> move to the anode to give up electrons and become oxygen gas (O<sub>2</sub>), chlorine gas (Cl<sub>2</sub>), hypochlorite ion (OCl<sup>-</sup>), hypochlorous acid (HOCl) and hydrochloric acid, while positively charged ions such as H<sup>+</sup> and Na<sup>+</sup> move to the cathode to take up electrons and become hydrogen gas (H<sub>2</sub>) and sodium hydroxide (NaOH). The solution dissociates into an acidic solution from the anode (pH 2 to 3; oxidation-reduction potential (ORP) >1100 mV; active chlorine content (ACC) 10-90 ppm), and a basic solution from the cathode (pH 10 to 13; ORP -800 to -900 mV). The solution from the anode is called acidic electrolyzed water (AEW), acid oxidizing water (AOW), or electrolyzed oxidizing water (EOW), whereas the cathodic solution is known as basic electrolyzed water (BEW), alkaline electrolyzed water (ALEW), or electrolyzed reducing water (ERW). Neutral electrolyzed water (NEW; pH 7 to 8; ORP 750 mV) is produced by mixing the anodic solution with OH<sup>-</sup> ions or by using a single-cell chamber (5, 21, 22, 39, 109).

Various EW-producing machines are available in the market. Japan is currently the principal manufacturer of such machines (5). Generally, machines can be divided into those containing a diaphragm producing AEW and BEW (two-cell chamber),

and those without a septum producing NEW (single-cell chamber). The physical properties and chemical composition of EW varies dependent on concentration of NaCl, amperage level, time of electrolysis, or water flow rate (47). Based on their control systems, machines allow the users to select (i) birne flow rate, (ii) amperages and/or voltages, or (iii) a preset chlorine concentration level.

**General application.** AEW exerts strong antimicrobial properties against a variety of microorganisms. It may be used in a wide range of application areas such as medicine (treatment of wounds, disinfection of medical equipment and surfaces), dentistry, agriculture, livestock management, aquaculture or the food industries. BEW is mostly used as cleanser and degreaser before treatment with disinfecting agents (7, 15, 27, 52, 57). BEW also exerts a strong reducing potential responsible for the reduction of free radicals (5). In some applications, pre-treatment with BEW, followed by the application of AEW, was more effective than AEW treatment only. Pre-treatment with BEW seems to sensitize bacterial cell surfaces to the exposure to a disinfecting agent. NEW on the other hand is less frequently used than AEW, but has the advantage of being less corrosive and having a longer shelf life (21, 76). Hence, NEW may be an alternative to AEW under certain circumstances (22, 39, 109).

**Antimicrobial activity of AEW.** Scientists are arguing if pH, chlorine compounds, ORP, or combinations of these factors are responsible for the antimicrobial activity of AEW. Altogether, the presence of chlorine and a high ORP seem to be the main contributors to the antimicrobial activity of AEW (5).

The low pH of AEW is believed to reduce the bacterial growth and to raise the sensitivity of bacterial cells to active chlorine by sensitizing their outer membrane to the entry of HOCl (85). The different active chlorine compounds are considered to destroy the membranes of microorganisms, but different other modes of chlorine

action (e.g. decarboxylation of amino acids, reactions with nucleic acids, unbalanced metabolism after the destruction of key enzymes) have also been proposed (47, 53, 71, 72). Studies suggest that hypochlorous acid (HOCl) is the most active of the chlorine compounds (55, 71, 72). HOCl penetrates cell membranes and produces hydroxyl radicals acting on the microorganisms. These compounds exert their antimicrobial activity through the oxidation of key metabolic systems. The relative fractions of chlorine compounds ( $\text{Cl}_2$ , HOCl, and  $\text{OCl}^-$ ) are pH-dependant and they affect the bactericidal activity of AEW (25, 41, 63, 72, 85). The highest proportion of HOCl and maximal efficiency of AEW in inactivating bacteria was found at a pH of about 4.0 to 5.0. On the other hand, more  $\text{Cl}_2$  was present at lower pHs and more  $\text{OCl}^-$  at higher pHs. The bactericidal activity of AEW and ORP increase with active chlorine concentrations indicating that chlorine is a strong oxidizing agent (85). Complete inactivation of *Escherichia (E.) coli* O157:H7 and *Listeria (L.) monocytogenes* was reported at ACCs of 2 ppm or above, regardless of pH (85).

By some authors, the high ORP is believed to be the determining factor for the antimicrobial activity of AEW (4, 41, 65, 106). Al-Haq et al. (5) reported that inactivation of *E. coli* was primarily dependent on ORP and not on residual chlorine. The ORP of a solution is an indicator of its ability to oxidize or reduce, with higher ORP values corresponding to greater oxidizing strength. The high ORP of AEW may be due to the oxygen released by the rupture of the weak and unstable bond between the hydroxy and chloric radicals (5). Moreover, the high ORP probably changes the electron flow in the cells. Oxidation due to the high ORP of AEW may damage cell membranes, cause the oxidation of sulfhydryl compounds on cell surfaces, and create disruption in cell metabolic processes leading to the inactivation of bacterial cells (64, 65). Basically, the high ORP and low pH of AEW seem to react synergistic with HOCl

to inactivate microorganisms (11, 65, 85, 87). On the other hand, complete loss of bactericidal activity was observed when ORP decreased to less than 848 mV (99).

**Influence factors on the antimicrobial activity of AEW.** A limiting factor for the use of AEW is its loss of activity with time due to chlorine loss and ensuing HOCl decomposition (53, 62). When stored under open conditions, AEW rapidly loses its residual chlorine due to Cl<sub>2</sub> evaporation (5). Len et al. (62) observed a total chlorine loss within 100 h of storage. Under closed conditions, chlorine loss occurs due to self-decomposition but it is slower than under open conditions. Chlorine loss by decomposition can be enhanced by exposition to diffused light and agitation (62). As mentioned, the ratio of Cl<sub>2</sub> among chlorine compounds is pH-dependant (63, 85). The lower the pH, the more Cl<sub>2</sub> exist, which can easily volatilize. Theoretically, almost no chlorine loss occurs at a pH of 9 (62).

Furthermore, temperature, agitation, and the contact with organic compounds influence the antimicrobial activity of AEW. At higher temperatures, cell membranes of gram-negative bacteria become more fluidal and AEW enters the cells faster (7, 24). Low storage temperatures seem to stabilize residual chlorine and ORP (24). When AEW treatment was combined with agitation, higher reductions were observed (87). Probably, cells removed from the surfaces during agitation were immediately inactivated by AEW (5, 87). Moreover, agitation might have facilitated the penetration of AEW into the remaining cell layers, or the well-mixed AEW allowed chlorine to react with cells more efficiently. On the other hand, the presence of organic matter reduced ACCs and ORPs rapidly (8, 82). Chlorine compounds react with proteins to form organo-chloramines, which exert a much smaller antimicrobial activity than free chlorine.



**Advantages and disadvantages of AEW.** AEW is environment friendly since it is generated by electrolysis of only water and a dilute salt solution (41, 50, 87). After use, AEW reverts to normal water (5, 13). Hence there is no need of handling, storing, or transportation of concentrated chemicals, which present a potential health hazard (5). Due to its nonselective antimicrobial properties, AEW does not lead to the development of resistances (5, 108). The use of AEW on different food commodities (e.g. produce and fish) did not negatively affect the organoleptic properties as color, scent, flavor, or texture (2, 5, 33, 34, 43, 48, 71). Moreover, many types of EW-producing machines allow EW to be produced on site and operational costs are low since only salt is needed to generate the sanitizer (5, 13).

Despite the listed advantages, some disadvantages associated with the application of AEW must be considered: (i) the initial costs for the purchase of the equipment may be high (5); (ii) some machines may form chlorine gas and cause discomfort for the operator (3, 4), (iii) AEW might be corrosive, irritating for hands, and phytotoxic due to its high ORP or free chlorine (31, 62, 76, 94); and (iv) the antimicrobial activity may be reduced by the presence of organic matter or inappropriate storage (8, 13, 54, 82, 95).

## **ANTIMICROBIAL ACTIVITY OF EW AGAINST MICROORGANISMS IN SUSPENSION**

The antimicrobial activity of AEW and NEW against various microorganisms is shown in Table 1. Generally, reductions of >6.0 log CFU/ml were reported for a variety of bacteria. The effectiveness of EW for reducing microorganisms is influenced by several factors such as type of EW (AEW, NEW), ACC, exposure time, treatment temperature, pH, amperage, or voltage. Because conditions vary among the studies, comparison of the results is often hampered. Fenner et al. (28) found

marked differences in the sensitivity to AEW between different bacterial species: *Proteus mirabilis* and *Staphylococcus (S.) aureus* were more sensitive to AEW than *Mycobacterium avium* ssp. *avium*, *Pseudomonas aeruginosa*, or *Enterococcus faecium*.

To be considered as effective, a sanitizer applied for 0.5 min must reduce microbial populations in suspension or in a biofilm at least by five or three orders of magnitude, respectively (8, 12, 21, 66, 75, 97, 105). By the use of AEW and NEW against suspended vegetative bacterial cells, these demands were met in most instances (Table 1). Spores, especially *Bacillus* spores, required longer exposure times than vegetative cells to obtain reductions >5.0 log CFU/ml (40, 108).

Venkitanarayanan et al. (106) showed that exposure to AEW reduced *E. coli* O157:H7 by >8.0 log CFU/ml within 5 min. At higher temperatures (35°C and 45°C), *E. coli* O157:H7 were inactivated at comparable levels within shorter exposure time. Compared with other studies, the relatively high ACC is noteworthy (Table 1). Moreover, Venkitanarayanan et al. (106) reported that AEW treatment reduced *Salmonella* Enteritidis from 7.8 log CFU/ml to non-detectable levels within 10 min and to less than 1.0 log CFU/ml within 5 min. For *Campylobacter jejuni* and different *Vibrio* species, already an AEW exposure for a few seconds yielded reductions of >6.5 log CFU/ml (84, 90). By the use of NEW for 5 min (ACC ranging from 60 to 93 ppm), *E. coli* O157:H7 were reduced from 7.5 log CFU/ml to non-detectable levels and *Salmonella* Enteritidis were reduced by >6.0 log CFU/ml (20, 21).

Similar to the inactivation of *E. coli* O157:H7 and *Salmonella* Enteritidis, Venkitanarayanan et al. (106) observed reductions of *L. monocytogenes* by >7.0 log CFU/ml after the application of AEW (Table 1). By the use of AEW with a slightly increased ACC, *L. monocytogenes* were reduced by 9.2 log CFU/ml within a few seconds (40), whereas NEW (ACC of 60 ppm) yielded reductions of >7.0 log CFU/ml within 5 min (20, 21).

*S. aureus* is involved in a wide variety of infections, and some strains producing staphylococcal enterotoxins (SE) are also responsible for food-borne intoxications. Park et al. (87) observed reductions of *S. aureus* by >9.0 log CFU/ml within 0.5 min (Table 1). Decreasing ACCs to 10 ppm yielded reductions of only 4.0 log CFU/ml. Fenner et al. (28) reported a reduction of *S. aureus* populations (8.0 log CFU/ml) to non-detectable levels within 5 min, whereas Vorobjeva et al. (108) obtained the same reductions within 0.5 min. By the use of NEW with increased ACCs, *S. aureus* were also reduced by >7.0 log CFU/ml within 5 min (21). Interestingly, results of Suzuki et al. (102) suggested that AEW is able to inactivate the staphylococcal enterotoxin SEA by cleaving it into peptid fragments.

Spores are generally less sensitive than vegetative cells to disinfecting agents including AEW (Table 1). To reduce *Bacillus cereus* spores by 3.5 orders of magnitudes, an exposure time of 2 min was required, whereas vegetative cells were reduced by 8.0 log CFU/ml within 0.5 min (40). However, by the use of AEW containing 43 ppm of active chlorine for 5 min, reductions by more than six orders of magnitude were noted for both vegetative cells and spores (108). Otherwise, an exposure time of 15 min was required to inactivate an initial count of 1'000 *Aspergillus parasiticus* spores by AEW containing 20 to 30 ppm of active chlorine (103). Interestingly, the results suggested that AEW might be able to eliminate the mutagenicity of aflatoxin AFB<sub>1</sub> by the effect of hydroxyl radicals originating from HOCl.

Researchers also confirmed AEW to be effective against blood-borne viruses including hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) (46, 74, 93, 104). In view of food-borne viral infections, further investigations are required to evaluate the use of AEW in this context.

## ANTIMICROBIAL ACTIVITY OF EW AGAINST MICROORGANISMS ON SURFACES AND UTENSILS

Surfaces and utensils present important sources for direct or indirect contamination of food products with pathogenic and spoilage microorganisms. In relation to the amount of organic residues present on surfaces, ACCs and the antimicrobial activity of AEW is reduced (8, 82). Ayebah et al. (8) recommend the sequential treatment with BEW and AEW. BEW may remove food residues and makes the adherent bacteria more susceptible to AEW. On the other hand, AEW seems to be effective to prevent cross-contamination (37, 38, 43, 57, 87).

**Cutting boards.** Venkitanarayanan et al. (107) examined the efficiency of AEW with different temperatures and ACCs in inactivating *E. coli* O157:H7 and *L. monocytogenes* on plastic cutting boards. The highest reductions were obtained for *E. coli* O157:H7 after treatment at 35°C for 20 min, 45°C for 10 min or 55°C for 5 min, and for *L. monocytogenes* at 35°C for 10 min (Table 2). *Vibrio parahaemolyticus* were reduced from 5.8 to less than 1.0 log CFU/cm<sup>2</sup> after 1 min of exposure to AEW (18). By rinsing plastic cutting boards with NEW, *E. coli*, *S. aureus*, *Pseudomonas aeruginosa*, and *L. monocytogenes* were reduced by about five orders of magnitude (22).

Wooden cutting boards are considered more difficult to sanitize than plastic boards (1, 18). Due to its physical structure, wood is able to absorb moisture and to protect bacteria from disinfecting agents. On the other hand, certain wood species may contain endogenous antibacterial properties leading to the desiccation of bacteria as a result of their hygroscopic characteristics. Rinsing wooden cutting boards with NEW for 1 min reduced populations of *E. coli*, *S. aureus*, *Pseudomonas aeruginosa*, and *L. monocytogenes* by less than three orders of magnitude (22).

Extending the exposure time to 5 min yielded reductions of about four orders of magnitude (Table 2). No significant differences were found between the application of AEW and distilled water in inactivating *Vibrio parahaemolyticus* on bamboo cutting boards (18). Bamboo may contain substances that interact with chlorine-based compounds and neutralize the antibacterial activity.

**Processing gloves.** Liu and Su (68) analyzed the effects of AEW on reusable and disposable gloves (natural rubber latex, natural latex, nitrile) and on clean and soil-containing gloves. *L. monocytogenes* were completely inactivated on each glove type after 5 min of treatment (Table 2). Longer survival of *L. monocytogenes* was observed in the presence of organic matter (Table 3).

**Stainless steel, tiles, glass, vitreous china.** On stainless steel, application of AEW for 5 min yielded reductions by 1.8 to 3.7 orders of magnitude (Table 2). Populations of *Vibrio parahaemolyticus* were reduced by more than 5.0 log CFU/cm<sup>2</sup> within only 0.5 min (18). In the presence of organic matter (crab meat residues), *L. monocytogenes* were reduced by 2.3 orders of magnitude (Table 3). By the use of NEW for 1 min, *E. coli* O157:H7, *L. monocytogenes*, *Pseudomonas aeruginosa*, and *S. aureus* were reduced by more than six orders of magnitude (Table 2). High reductions were also obtained for these pathogens on glass (21).

On tiles, application of AEW for 5 min yielded reductions by 1.8 to 4.2 orders of magnitude (Table 2). Populations of *Vibrio parahaemolyticus* were reduced by more than 5.0 log CFU/cm<sup>2</sup> within less than 1 min (18). In the presence of organic matter, *L. monocytogenes* were reduced by 1.5 to 2.3 orders of magnitude (Table 3). Results from vitreous china were comparable with those from stainless steel, tiles, or glass (Table 2). With agitation, *Enterobacter aerogenes* and *S. aureus* were reduced to non-detectable levels (3.0 log CFU/cm<sup>2</sup>) on vitreous china (87).

**Biofilms.** Biofilms are a structured community of bacterial cells enclosed in a self-producing polymeric matrix (glycocalyx), which constitutes a protected mode of growth on surfaces and allows survival in hostile environments. The higher resistance of bacteria in biofilms to sanitizers has been attributed to various factors as protection by the matrix, neutralization of the sanitizer, genetic modification of the cell wall, and slow uptake of antimicrobial agents (16, 19, 23, 100). Only limited data exist on the efficiency of EW in inactivating bacteria in biofilms.

Kim et al. (42) showed that AEW reduced *L. monocytogenes* in biofilms on stainless steel to non-detectable levels within 5 min (Table 2). The highest inactivation rate was reported within the first seconds of treatment. Thus AEW needed longer exposure times to reach the cells inside the biofilm. Depending on the treatment time, Ayebah et al. (7) reported reductions of *L. monocytogenes* by 4.3 to 5.2 orders of magnitude. The effectiveness of AEW with different chlorine concentrations (47 and 85 ppm) did thereby not differ significantly. Other studies also suggest the existence of a threshold concentration beyond which further increase does not enhance the effectiveness (60, 91). The reductions of *L. monocytogenes* in biofilms obtained in the presence of organic matter are shown in Table 3. Moreover, Ayebah et al. (7) obtained the highest reductions by sequential BEW and AEW treatment, even in the presence of organic matter. The higher efficiency of this sequential treatment was also reported by Koseki et al. (55, 57). Probably, BEW destabilized or dissolved the glycocalyx and thereby facilitated the penetration of the active AEW components.

**Abattoirs.** Bach et al. (9) compared the effectiveness of AEW and a common sanitizer (Mikrolene) for the use in abattoirs. After standard pre-cleaning, AEW turned out to be more effective in inactivating bacteria in different slaughterhouse areas. Within the slaughter of cattle, the contamination risk associated with the hide is of special interest. Both saprophytes and pathogens as *E. coli* O157:H7 might be

transferred to the carcasses during dehidung (6, 70, 73, 89). Besides the maintenance and optimization of slaughter hygiene practices, decontamination treatments for hides have been established (10, 49, 96). Bosilevac et al. (15) used a high-pressure spray treatment of BEW (52°C, 10 s, pH 11.2) and AEW (60°C, 10 s, pH 2.4, ACC 70 ppm) on cattle hides. Comparable to other hide treatments, total microbial counts and *Enterobacteriaceae* were reduced by 3.5 and 4.3 log CFU/100 cm<sup>2</sup>, respectively. However, the effect of this specific treatment was smaller in an earlier study (14).

### **ANTIMICROBIAL ACTIVITY OF EW AGAINST MICROORGANISMS IN PROCESS WATER**

Water washing is widely used for produce and minimally processed vegetables. Hence accumulation of microorganisms in the process water must be prevented (29). Ongeng et al. (81) investigated the effect of the electrolysis procedure in water used for the washing of vegetables. Thereby the antimicrobial activity against *Pseudomonas fluorescens*, *Pantoea agglomerans*, and *Rahnella aquatilis* was tested. Industrial process water, which showed higher microbial (8.0 log CFU/ml) and organic load than tap water, still had a microbial load of >6.0 log CFU/ml after electrolysis with the attainable amperage of 0.7 A (ACC of 1.1 ppm). If salt was supplemented (5 ml of 20% NaCl/10 l), the tested bacteria were reduced by about four orders of magnitude. By raising the amperage to 1.3 A, which generated ACCs above 2 ppm, complete inactivation was achieved. Moreover, AEW produced with tap water had a stronger antimicrobial activity than AEW produced with process water (81).

### **ANTIMICROBIAL ACTIVITY OF EW AGAINST MICROORGANISMS ON FOOD PRODUCTS**

The antimicrobial activities of AEW or NEW on various food products are shown in Table 4 and 5. Moreover, the effects of sequential BEW and AEW treatment are summarized in Table 6.

**Vegetables and fruits.** On strawberries, AEW treatment for 10 min achieved a reduction of naturally present aerobic bacteria, coliforms, and fungi by 1.6, 2.4, and 1.6 log CFU/strawberry to non-detectable levels, respectively (56). Similar reductions were also obtained on cucumbers (Table 4). The combined treatment with BEW and AEW yielded higher reductions for cucumbers, but not for strawberries (Table 6). The latter is in agreement with former studies (56, 69, 112). Probably due to the complex surface structure of strawberries, longer exposure times were required to allow sanitizers to infiltrate the surface. On tomatoes, AEW reduced *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* Enteritidis by about 7.5 log CFU/tomato (11).

After application of AEW containing only 3.6 ppm of active chlorine on lettuce, Ongeng et al. (81) observed reductions of *Enterobacteriaceae*, lactic acid bacteria, and psychrotrophs by 2.6, 1.9, and 3.3 log CFU/g, respectively. Park et al. (86) reported similar reductions of *E. coli* O157:H7 (2.8 log CFU/leaf) and *L. monocytogenes* (2.4 log CFU/leaf) after AEW treatment (Table 4). Recently, AEW was shown to be as effective as chlorine in reducing pathogens (*E. coli* O157:H7, *Salmonella*, *L. monocytogenes*) on leafy greens (101). Thus AEW may be used as a suitable alternative to chlorine for the treatment of leafy greens.

In further study (57), the effects of temperature and BEW pre-treatment on the efficiency of AEW against *E. coli* O157:H7 and *Salmonella* on lettuce were examined (Table 4). Reductions obtained by AEW at 4°C or room temperature within 1 min were not higher than to those obtained by chlorinated water or distilled water. Rise of temperature (50°C) and/or exposure time (5 min) yielded higher reductions. BEW pre-treatment at room temperature for 5 min increased the reductions by about 0.5



orders of magnitude (Table 6). Highest reductions were obtained at a pre-treatment temperature of 50°C, regardless of duration or temperature of the AEW treatment (57). Moreover, Yang et al. (109) examined the effects of BEW and AEW (30°C, 5 min, pH 9 or 4; ORP -750 or 1150 mV; ACC 22 to 198 ppm) on biofilms attached to lettuce leaves. *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* Typhimurium were thereby reduced by about two orders of magnitude.

By the use of NEW for 5 min, *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* Typhimurium on lettuce were reduced by 3.0, 4.0, and 2.5 log CFU/g, respectively (109). Otherwise, NEW reduced *L. monocytogenes* and *Salmonella* Enteritidis on tomatoes by 4.3 to 4.9 log CFU/cm<sup>2</sup> (20). Moreover, NEW reduced aerobic bacteria on diced potatoes, radish shreds, carrot slices, and spinach leaves by 0.1 to 2.3 log CFU/g (Table 4). Thereby, rinsing was generally more effective than dipping (39).

**Fish and seafood.** On carp skin treated for 15 min with AEW, total microbial counts were reduced by 2.8 log CFU/cm<sup>2</sup> (Table 5). Pre-treatment with BEW yielded comparable results (Table 6). On tilapia skin immersed in AEW, higher reductions were obtained for *Vibrio parahaemolyticus* than for *E. coli* O157:H7 (37). On carp filets treated for 15 min with AEW, total microbial counts were reduced by 2.0 log CFU/g (72). The use of AEW on tuna filets yielded reductions of the natural microflora by about one order of magnitude (Table 5). Depending on exposure time and temperature, Ozer and Demirci (83) reported reductions of *E. coli* O157:H7 and *L. monocytogenes* on salmon filets ranging from 0.4 to 1.1 log CFU/g.

To investigate the antimicrobial effect of AEW on oysters, inoculated oysters were placed into tanks containing AEW (ACC of 30 ppm) and the AEW salt concentration was set at 1% (90). After four hours of exposition, *Vibrio parahaemolyticus* and *Vibrio vulnificus* were reduced by about one order of magnitude (Table 5). Further exposition did not increase the reductions. Probably due to the unfavorable growth

environment, oysters stopped water filtering and thereby hampered the entry of AEW (90).

**Carcasses, raw meat, and ready-to-eat meat.** Fabrizio et al. (27) compared the effect of AEW solutions for immersion and spray washing of chicken carcasses. Immersion of carcasses in AEW (4°C, 45 min) reduced aerobic bacteria, total coliforms, *E. coli*, and *Salmonella* Typhimurium by 0.8 to 1.3 log CFU/ml carcass rinsate (Table 5). Otherwise, reductions obtained by spray washing (15 s) with AEW and distilled water did not differ significantly. Spray washing with BEW followed by immersion in AEW (Table 6) yielded higher reductions (1.5 to 2.4 log CFU/ml). Spray treatment with BEW was as effective in removing fecal material as the commonly used trisodium phosphate (44). Moreover, the results of Hinton et al. (35) suggested that AEW treatment extended the shelf life of refrigerated poultry.

Kim et al. (44) investigated the effectiveness of AEW to reduce *Campylobacter jejuni* on chicken carcasses (Table 5). Reductions of 2.3 log CFU/g were obtained by immersion, but additional pre-spraying did not improve the efficiency. Spray treatment alone reduced *Campylobacter jejuni* by 1.1 log CFU/g. However, all treatments failed to completely eliminate *Campylobacter*. Furthermore, AEW reduced *Campylobacter jejuni* on fresh chicken wings by about three orders of magnitude and was thereby as effective as chlorine water (84). Gellynck et al., (30) analyzed the economics of reducing *Campylobacter* at different levels within the poultry meat chain (farm, processing plant, consumer). These authors found that the decontamination of carcasses with AEW in the processing plant was the most efficient (cost-benefit ratio) among the evaluated measures.

Fabrizio and Cutter (25) investigated the effectiveness of AEW spray treatment on pork bellies in order to reduce total microbial counts, *Campylobacter coli*, coliforms, *E. coli*, *L. monocytogenes*, and *Salmonella* Typhimurium (Table 5). Only the effect of AEW

against *Campylobacter* differed significantly from that obtained for distilled water (1.8 log CFU/cm<sup>2</sup>). On frankfurters and ham, spray treatment with AEW or a combined spray treatment with BEW and AEW failed to reduce *L. monocytogenes* by more than one order of magnitude (Table 5 and 6). Other tested sanitizing agents did also not achieve higher reductions (26). This might be due to the short contact times and the binding of chemicals by proteins. By dipping frankfurters in AEW for 15 min, *L. monocytogenes* were reduced by 1.5 log CFU/g (Table 5).

**Eggs.** Electrostatic spraying of shell eggs with AEW (hourly for one day) reduced *E. coli*, *S. aureus*, and *Salmonella* Typhimurium by three to six orders of magnitude (Table 5), whereas *L. monocytogenes* were reduced by 1.0 to 4.0 log CFU/egg (92). In another study, immersion of eggs in AEW for 5 min with agitation (100 rpm) reduced *L. monocytogenes* and *Salmonella* Enteritidis by 3.7 and 2.3 log CFU/egg, respectively (88). Pre-wash with BEW yielded reductions of  $\geq 3.0$  log CFU/egg within shorter exposure times (Table 6).

**Application of AEW as ice.** AEW may be applied as solution or ice. Frozen AEW was tested on lettuce and pacific saury (45, 51). The main antimicrobial effect of frozen AEW was attributed to the emitted Cl<sub>2</sub> (36, 50). Cl<sub>2</sub> emission in AEW-ice was proportional to the ACC before freezing (51). Because the boiling point of Cl<sub>2</sub> is -34°C, AEW-ice should be prepared at -40°C to prevent early chlorine loss.

On iceberg lettuce placed into containers with AEW-ice (pH 2.6), reductions of *L. monocytogenes* accounted for about 1.5 log CFU/g and no significant differences were found at ACCs of 40 and 70 ppm (51). The highest reductions of *E. coli* O157:H7 (2.5 log CFU/g) were obtained with AEW-ice containing 240 ppm of active chlorine. However, this ACC caused physiological disorder resembling leaf burn. AEW-ice with ACCs of 40 and 70 ppm did not affect the color of lettuce and still reduced *E.*

*coli* O157:H7 by one order of magnitude. To achieve reductions of both pathogens by at least 1.5 log CFU/g, ten times the weight of AEW-ice relative to the weight of lettuce was required. The best results were obtained after an exposure time of 120 min. Extension of this time did not lead to further reductions. AEW-ice may serve simultaneously for refrigeration and control of pathogens (51).

In another study, AEW-ice (pH 5.1; ACC of 47 ppm) was used on pacific saury to extend shelf life, to suppress lipid oxidation and the formation of volatile basic nitrogen, and to retard the accumulation of alkaline compounds (45). In this study, the storage of saury in tap water ice and AEW-ice were compared. Hence the growth of aerobic bacteria and psychrotrophs was slower and growth of coliforms did not occur when saury was stored using AEW-ice.

### **IMPACT OF EW APPLICATION FOR THE FOOD INDUSTRY**

AEW treatment may be used as a method for inactivating food-borne pathogens and reducing microbial contamination on processing surfaces and various food products (e.g. vegetables and fruits). However, microbial reductions on surfaces and especially food products were less distinct than those obtained in suspension. In particular, the adverse effect of organic matter on the antimicrobial activity of AEW must be considered for the use of this technology in the food industry.

On some food commodities, treatment with BEW followed by AEW showed stronger activity than treatment with AEW only. Interestingly, sequential BEW and AEW treatment also yielded highest reductions in *L. monocytogenes* biofilms on stainless steel, even in the presence of organic matter. Hence combination of AEW with other preservative agents should be further evaluated.

The EW technology deserves consideration in discussing possibilities for sanitizing of equipment or for decontamination of certain food products.

Nevertheless, decontamination treatments for food products should always be seen as a part of an integral food safety system. In particular, such treatments cannot replace strict adherence to good manufacturing and hygiene practices on all stages of the food production process.

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**FIGURE LEGEND**

FIGURE 1. Schematics of electrolyzed water generation. The basic chemical reactions at the anode can be summarized as follows:  $2\text{H}_2\text{O} \rightarrow 4\text{H}^+ + \text{O}_2\uparrow + 4\text{e}^-$ ;  $2\text{NaCl} \rightarrow \text{Cl}_2\uparrow + 2\text{e}^- + 2\text{Na}^+$ ;  $\text{Cl}_2 + \text{H}_2\text{O} \rightarrow \text{HCl} + \text{HOCl}$ . At the cathode, the main chemical reactions comprise:  $2\text{H}_2\text{O} + 2\text{e}^- \rightarrow 2\text{OH}^- + \text{H}_2\uparrow$ ;  $2\text{NaCl} + 2\text{OH}^- \rightarrow 2\text{NaOH} + \text{Cl}^-$ .

TABLE 1. Antimicrobial activity of AEW and NEW against microorganisms in suspension

| Microorganisms                  | EW  | Reduction<br>(log CFU ml <sup>-1</sup> ) | Temperature<br>(°C) | Exposure<br>time (min) | pH  | ORP<br>(mV) | Active chlorine<br>(ppm) | Reference |
|---------------------------------|-----|--|---------------------|------------------------|-----|-------------|--------------------------|-----------|
| <i>Aeromonas liquefaciens</i>   | AEW | >7.0                                     | na <sup>a</sup>     | 0.5                    | 2.8 | 1125        | 43                       | 108       |
| <i>Alcaligenes faecalis</i>     | AEW | >7.0                                     | na                  | 0.5                    | 2.8 | 1125        | 43                       | 108       |
| <i>Bacillus</i> spp.            | AEW | 2.3                                      | 25                  | 1                      | 2.2 | na          | 40                       | 72        |
| <i>Bacillus cereus</i>          | AEW | 8.0                                      | 24                  | 0.5                    | 2.5 | 1123        | 10                       | 40        |
| Spores                          | AEW | 3.5                                      | 24                  | 2                      | 2.5 | 1123        | 10                       | 40        |
| Cells and spores                | AEW | >6.0                                     | na                  | 5                      | 2.8 | 1125        | 43                       | 108       |
| <i>Bacillus subtilis</i>        | AEW | >6.0                                     | na                  | 5                      | 2.2 | 1153        | 49                       | 47        |
| <i>Campylobacter jejuni</i>     | AEW | >7.0                                     | 23                  | 0.2                    | 2.6 | 1082        | 50                       | 84        |
| <i>Citrobacter freundii</i>     | AEW | >7.0                                     | na                  | 0.5                    | 2.8 | 1125        | 43                       | 108       |
| <i>Enterobacter aerogenes</i>   | AEW | >9.0                                     | 23                  | 0.5                    | 2.8 | 1163        | 25                       | 87        |
| <i>Enterobacteriaceae</i>       | AEW | >6.0                                     | na                  | 1                      | 2.2 | na          | 40                       | 72        |
| <i>Enterococcus faecium</i>     | AEW | >8.0                                     | na                  | 0.5                    | 2.8 | 1125        | 43                       | 108       |
|                                 | AEW | 8.0                                      | 22                  | 15                     | 3.0 | 1100        | 40                       | 28        |
|                                 | NEW | >6.0                                     | 25                  | 10                     | 6.5 | 850         | 20                       | 32        |
| <i>Escherichia coli</i>         | AEW | >8.0                                     | na                  | 0.5                    | 2.8 | 1125        | 43                       | 108       |
|                                 | NEW | >6.0                                     | 23                  | 5                      | 8.2 | 745         | 93                       | 20        |
|                                 | NEW | >6.0                                     | 25                  | 10                     | 6.5 | 850         | 20                       | 32        |
| <i>Escherichia coli</i> O157:H7 | AEW | 8.9                                      | 24                  | 0.2                    | 2.6 | 1160        | 56                       | 40        |
|                                 | AEW | >8.0                                     | 23                  | 5                      | 2.4 | 1155        | 82                       | 106       |
|                                 | AEW | 8.0                                      | 35                  | 2                      | 2.4 | 1155        | 82                       | 106       |
|                                 | AEW | 8.0                                      | 45                  | 1                      | 2.4 | 1155        | 82                       | 106       |
|                                 | AEW | >7.0                                     | 22                  | 1                      | 2.5 | 1130        | 45                       | 86        |
|                                 | NEW | >7.0                                     | 23                  | 5                      | 8.0 | >700        | 60                       | 21        |
| <i>Flavobacter</i> spp.         | AEW | >8.0                                     | na                  | 0.5                    | 2.8 | 1125        | 43                       | 108       |
|                                 | AEW | >6.0                                     | na                  | 1                      | 2.2 | na          | 40                       | 72        |

TABLE 1. *Continued*

| Microorganisms                               | EW  | Reduction<br>(log CFU ml <sup>-1</sup> ) | Temperature<br>(°C) | Exposure<br>time (min) | pH  | ORP<br>(mV) | Active chlorine<br>(ppm) | Reference |
|--|-----|--|---------------------|------------------------|-----|-------------|--------------------------|-----------|
| <i>Listeria monocytogenes</i>                | AEW | 9.2                                      | 24                  | 0.2                    | 2.6 | 1160        | 56                       | 40        |
|  | AEW | >8.0                                     | 23                  | 0.1                    | 2.5 | 1150        | 50                       | 67        |
|  | AEW | >7.0                                     | 22                  | 1                      | 2.5 | 1130        | 45                       | 86        |
|  | AEW | >7.0                                     | 4                   | 10                     | 2.6 | 1158        | 48                       | 106       |
|  | AEW | >7.0                                     | 23                  | 5                      | 2.6 | 1158        | 48                       | 106       |
|  | AEW | >7.0                                     | 35                  | 2                      | 2.6 | 1158        | 48                       | 106       |
|  | AEW | >7.0                                     | 45                  | 1                      | 2.6 | 1158        | 48                       | 106       |
|  | AEW | >6.0                                     | na                  | 1                      | 2.4 | 1170        | 44                       | 8         |
|  | NEW | >7.0                                     | 23                  | 5                      | 8.0 | >700        | 60                       | 21        |
|  | NEW | >6.0                                     | 25                  | 10                     | 6.5 | 850         | 20                       | 32        |
| <i>Mycobacterium avium</i> ssp. <i>avium</i> | AEW | 8.0                                      | 22                  | 15                     | 3.0 | 1100        | 40                       | 28        |
| <i>Proteus mirabilis</i>                     | AEW | 8.0                                      | 22                  | 5                      | 3.0 | 1100        | 40                       | 28        |
| <i>Proteus vulgaris</i>                      | AEW | >8.0                                     | na                  | 0.5                    | 2.8 | 1125        | 43                       | 108       |
| <i>Pseudomonas aeruginosa</i>                | AEW | >8.0                                     | na                  | 0.5                    | 2.8 | 1125        | 43                       | 108       |
|  | AEW | 8.0                                      | 22                  | 30                     | 3   | 1100        | 40                       | 28        |
|  | AEW | >6.0                                     | na                  | 5                      | 2.2 | 1153        | 49                       | 47        |
|  | NEW | >7.0                                     | 23                  | 5                      | 8.0 | >700        | 60                       | 21        |
| <i>Salmonella</i> Enteritidis                | AEW | >7.0                                     | 23                  | 5                      | 2.4 | 1151        | 82                       | 106       |
|  | NEW | >6.0                                     | 23                  | 5                      | 8.2 | 745         | 93                       | 20        |
| <i>Salmonella</i> Typhimurium                | NEW | >6.0                                     | 25                  | 10                     | 6.5 | 850         | 20                       | 32        |
| <i>Staphylococcus aureus</i>                 | AEW | >9.0                                     | 23                  | 0.5                    | 2.8 | 1163        | 25                       | 87        |
|  | AEW | >8.0                                     | na                  | 0.5                    | 2.8 | 1125        | 43                       | 108       |
|  | AEW | 8.0                                      | 22                  | 5                      | 3.0 | 1100        | 40                       | 28        |
|  | AEW | 4.1                                      | 23                  | 0.5                    | 3.2 | 1116        | 10                       | 84        |
|  | NEW | >7.0                                     | 23                  | 5                      | 8.0 | >700        | 60                       | 21        |
|  | NEW | >6.0                                     | 25                  | 10                     | 6.5 | 850         | 20                       | 32        |

TABLE 1. *Continued*

| Microorganisms                        | EW  | Reduction<br>(log CFU ml <sup>-1</sup> ) | Temperature<br>(°C) | Exposure<br>time (min) | pH  | ORP<br>(mV) | Active chlorine<br>(ppm) | Reference |
|---------------------------------------|-----|--|---------------------|------------------------|-----|-------------|--------------------------|-----------|
| <i>Vibrio parahaemolyticus</i>        | AEW | >6.6                                     | na                  | 0.3                    | 3.2 | 1104        | 10                       | 90        |
| <i>Vibrio vulnificus</i>              | AEW | >6.6                                     | na                  | 0.3                    | 3.2 | 1104        | 10                       | 90        |
| <i>Aspergillus parasiticus</i> spores | AEW | 3.0                                      | na                  | 15                     | 2.5 | 1164        | 20 to 30                 | 103       |
| <i>Candida albicans</i>               | AEW | 8.0                                      | 22                  | 5                      | 3.0 | 1100        | 40                       | 28        |
| <i>Penicillium expansum</i> spores    | AEW | 4.0                                      | na                  | 5                      | 3.5 | 1027        | 18                       | 79        |
|                                       | AEW | 4.8                                      | na                  | 0.5                    | 3.1 | 1133        | 60                       | 80        |

<sup>a</sup> na, not available.



TABLE 2. Antimicrobial activity of AEW and NEW on surfaces and utensils

| Material / surface | Microorganisms                  | EW  | Reduction (log)         | Temperature (°C) | Exposure time (min) | pH  | ORP (mV) | Active chlorine (ppm) | Reference |
|--------------------|---------------------------------|-----|-------------------------|------------------|---------------------|-----|----------|-----------------------|-----------|
| Ceramic tile       | Aerobic bacteria                | AEW | 2.4/cm <sup>2</sup>     | na <sup>a</sup>  | 1                   | 2.6 | 1156     | 55                    | 37, 38    |
|                    | <i>Enterobacter aerogenes</i>   | AEW | 2.2/cm <sup>2</sup>     | 23               | 5                   | 2.6 | 1181     | 53                    | 87        |
|                    | <i>Staphylococcus aureus</i>    | AEW | 1.8/cm <sup>2</sup>     | 23               | 5                   | 2.6 | 1181     | 53                    | 87        |
|                    | <i>Vibrio parahaemolyticus</i>  | AEW | >5.0/cm <sup>2</sup>    | na               | 0.8                 | 2.7 | 1151     | 40                    | 18        |
| Ceramic tile chips | <i>Listeria monocytogenes</i>   | AEW | 4.2/25 cm <sup>2</sup>  | na               | 5                   | 2.5 | 1150     | 50                    | 67        |
| Cutting boards     |                                 |     |                         |                  |                     |     |          |                       |           |
| Bamboo             | <i>Vibrio parahaemolyticus</i>  | AEW | 3.5/cm <sup>2</sup>     | na               | 5                   | 2.7 | 1151     | 40                    | 18        |
| Plastic            | <i>Escherichia coli</i>         | NEW | 5.0/50 cm <sup>2</sup>  | na               | 1                   | 7.8 | 775      | 64                    | 22        |
|                    | <i>Escherichia coli</i> O157:H7 | AEW | 8.0/100 cm <sup>2</sup> | 35               | 20                  | 2.6 | 1162     | 90                    | 107       |
|                    |                                 |     |                         | 45               | 10                  | 2.5 | 1157     | 93                    | 107       |
|                    |                                 |     |                         | 55               | 5                   | 2.3 | 1147     | 45                    | 107       |
|                    | <i>Listeria monocytogenes</i>   | NEW | 5.0/50 cm <sup>2</sup>  | na               | 1                   | 7.8 | 775      | 64                    | 22        |
|                    |                                 | AEW | 5.3/100 cm <sup>2</sup> | 35               | 10                  | 2.4 | 1156     | 66                    | 107       |
|                    | <i>Pseudomonas aeruginosa</i>   | NEW | 5.0/50 cm <sup>2</sup>  | na               | 1                   | 7.8 | 775      | 64                    | 22        |
|                    | <i>Staphylococcus aureus</i>    | NEW | 5.0/50 cm <sup>2</sup>  | na               | 1                   | 7.8 | 775      | 64                    | 22        |
|                    | <i>Vibrio parahaemolyticus</i>  | AEW | >5.0/cm <sup>2</sup>    | na               | 1                   | 2.7 | 1151     | 40                    | 18        |
| Wood               | <i>Escherichia coli</i>         | NEW | 4.0/50 cm <sup>2</sup>  | na               | 5                   | 7.8 | 775      | 64                    | 22        |
|                    | <i>Listeria monocytogenes</i>   | NEW | 4.0/50 cm <sup>2</sup>  | na               | 5                   | 7.8 | 775      | 64                    | 22        |
|                    | <i>Pseudomonas aeruginosa</i>   | NEW | 4.0/50 cm <sup>2</sup>  | na               | 5                   | 7.8 | 775      | 64                    | 22        |
|                    | <i>Staphylococcus aureus</i>    | NEW | 4.0/50 cm <sup>2</sup>  | na               | 5                   | 7.8 | 775      | 64                    | 22        |
|                    | <i>Vibrio parahaemolyticus</i>  | AEW | 5.7/cm <sup>2</sup>     | na               | 5                   | 2.7 | 1151     | 40                    | 18        |

TABLE 2. *Continued*

| Material / surface | Microorganisms                  | EW  | Reduction (log)            | Temperature (°C)        | Exposure time (min) | pH  | ORP (mV) | Active chlorine (ppm) | Reference |    |
|--------------------|---------------------------------|-----|----------------------------|-------------------------|---------------------|-----|----------|-----------------------|-----------|----|
| Glass              | <i>Enterobacter aerogenes</i>   | AEW | 2.2/cm <sup>2</sup>        | 23                      | 5                   | 2.6 | 1181     | 53                    | 87        |    |
|                    | <i>Escherichia coli</i> O157:H7 | NEW | >6.0/50 cm <sup>2</sup>    | 23                      | 1                   | 8.0 | >700     | 60                    | 21        |    |
|                    | <i>Listeria monocytogenes</i>   | NEW | >6.0/50 cm <sup>2</sup>    | 23                      | 1                   | 8.0 | >700     | 60                    | 21        |    |
|                    | <i>Pseudomonas aeruginosa</i>   | NEW | >6.0/50 cm <sup>2</sup>    | 23                      | 1                   | 8.0 | >700     | 60                    | 21        |    |
|                    | <i>Staphylococcus aureus</i>    | NEW | >6.0/50 cm <sup>2</sup>    | 23                      | 1                   | 8.0 | >700     | 60                    | 21        |    |
|                    |                                 |     | AEW                        | 1.7/cm <sup>2</sup>     | 23                  | 5   | 2.6      | 1181                  | 53        | 87 |
| Gloves             | <i>Listeria monocytogenes</i>   | AEW | 4.5 to 5.5/cm <sup>2</sup> | 23                      | 5                   | 2.6 | 1125     | 40                    | 68        |    |
| Stainless steel    | <i>Enterobacter aerogenes</i>   | AEW | 2.4/cm <sup>2</sup>        | 23                      | 5                   | 2.6 | 1181     | 53                    | 87        |    |
|                    | <i>Escherichia coli</i> O157:H7 | NEW | >6.0/50 cm <sup>2</sup>    | 23                      | 1                   | 8.0 | >700     | 60                    | 21        |    |
|                    | <i>Listeria monocytogenes</i>   | AEW | 3.7/25 cm <sup>2</sup>     | na                      | 5                   | 2.5 | 1150     | 50                    | 67        |    |
|                    |                                 |     | NEW                        | >6.0/50 cm <sup>2</sup> | 23                  | 1   | 8.0      | >700                  | 60        | 21 |
|                    | <i>Listeria monocytogenes</i>   |     |                            |                         |                     |     |          |                       |           |    |
|                    | Biofilms                        | AEW | 4.3/10 cm <sup>2</sup>     | 24                      | 0.5                 | 2.4 | 1163     | 47                    | 7         |    |
|                    | Biofilms                        | AEW | 5.2/10 cm <sup>2</sup>     | 24                      | 2                   | 2.4 | 1163     | 47                    | 7         |    |
|                    | Biofilms                        | AEW | 5.8/83 cm <sup>2</sup>     | 23                      | 0.2                 | 2.6 | 1160     | 56                    | 42        |    |
|                    | Biofilms                        | AEW | >10/83 cm <sup>2</sup>     | 23                      | 5                   | 2.6 | 1160     | 56                    | 42        |    |
|                    | <i>Pseudomonas aeruginosa</i>   | NEW | >6.0/50 cm <sup>2</sup>    | 23                      | 1                   | 8.0 | >700     | 60                    | 21        |    |
|                    | <i>Staphylococcus aureus</i>    | AEW | 1.8/cm <sup>2</sup>        | 23                      | 5                   | 2.6 | 1181     | 53                    | 87        |    |
|                    |                                 |     | NEW                        | >6.0/50 cm <sup>2</sup> | 23                  | 1   | 8.0      | >700                  | 60        | 21 |
|                    | <i>Vibrio parahaemolyticus</i>  | AEW | >5.0/cm <sup>2</sup>       | na                      | 0.5                 | 2.7 | 1151     | 40                    | 18        |    |
| Vitreous china     | <i>Enterobacter aerogenes</i>   | AEW | 2.3/cm <sup>2</sup>        | 23                      | 5                   | 2.6 | 1181     | 53                    | 87        |    |
|                    | <i>Staphylococcus aureus</i>    | AEW | 1.9/cm <sup>2</sup>        | 23                      | 5                   | 2.6 | 1181     | 53                    | 87        |    |

<sup>a</sup> na, not available

TABLE 3. *Antimicrobial activity of AEW against Listeria monocytogenes in the presence of organic matter/food residues*

| Material  | Reduction (log)              | Temperature (°C) | Exposure time (min) | pH  | ORP (mV) | Active chlorine (ppm) | Reference |
|---|------------------------------|------------------|---------------------|-----|----------|-----------------------|-----------|
| Ceramic tiles with crab meat residues   | 2.3/25cm <sup>2</sup>        | na <sup>a</sup>  | 5                   | 2.5 | 1150     | 50                    | 67        |
| Floor tiles with crab meat residues   | 1.5/25cm <sup>2</sup>        | na               | 5                   | 2.5 | 1150     | 50                    | 67        |
| Processing gloves with cooked shrimp meat diluted with distilled water              | 1.6 to 3.8/16cm <sup>2</sup> | 24               | 5                   | 2.6 | 1125     | 40                    | 68        |
| Stainless steel (biofilm), chicken serum added to the treatment solution (5 ml/l)   | 2.7/10cm <sup>2</sup>        | 24               | 0.5                 | 2.3 | 1166     | 44                    | 8         |
| Stainless steel (biofilm), chicken serum added to the treatment solution (7.5 ml/l) | 2.0/10cm <sup>2</sup>        | 24               | 0.5                 | 2.3 | 1166     | 44                    | 8         |
| Stainless steel (biofilm), chicken serum added to the treatment solution (7.5 ml/l) | >4.0/cm <sup>2</sup>         | 24               | 1                   | 2.3 | 1166     | 44                    | 8         |
| Stainless steel with crab meat residues   | 2.3/25cm <sup>2</sup>        | na               | 5                   | 2.5 | 1150     | 50                    | 67        |

<sup>a</sup> na, not available;

TABLE 4. Antimicrobial activity of AEW and NEW on fruits and vegetables

| Food products                 | Microorganisms   | EW     | Reduction (log) | Temperature (°C) | Exposure time (min) | pH   | ORP (mV)  | Active chlorine (ppm) | Reference |
|-------------------------------|--|--------|-----------------|------------------|---------------------|------|-----------|-----------------------|-----------|
| Carrots (slices)              | Aerobic bacteria   | NEW    | 1.0/g           | 23               | 3                   | 6.8  | na        | 20                    | 39        |
| Cucumbers                     | Aerobic bacteria   | AEW    | 1.5/cucumber    | na <sup>a</sup>  | 10                  | 2.6  | 1130      | 32.1                  | 56        |
|                               | Coliforms  | AEW    | 1.7/cucumber    | na               | 10                  | 2.6  | 1130      | 32.1                  | 56        |
|                               | Fungi  | AEW    | 1.7/cucumber    | na               | 10                  | 2.6  | 1130      | 32.1                  | 56        |
| Lettuce                       | Aerobic bacteria   | AEW    | 2.0/g           | na               | 5                   | 2.6  | 1140      | 30                    | 55        |
|                               | <i>Enterobacteriaceae</i>  | na     | 2.6/g           | na               | 5                   | na   | na        | 3.6                   | 81        |
|                               | <i>Enterococcus faecalis</i>                                       | NEW    | 2.6/ml          | 25               | 10                  | 6.5  | 850       | 50                    | 32        |
|                               | <i>Escherichia coli</i>  | NEW    | 0.2/ml          | 25               | 10                  | 6.5  | 850       | 50                    | 32        |
|                               | <i>Escherichia coli</i> O157:H7                                    | AEW    | 2.4/leaf        | 22               | 3                   | 2.5  | 1130      | 45                    | 86        |
|                               |  | NEW    | 3.0/g           | 30               | 5                   | 7    | >750      | 22 to 198             | 109       |
|                               | <i>Escherichia coli</i> O157:H7 and <i>Salmonella</i> <sup>b</sup> | AEW    | 0.6 to 0.9/g    | 4 or 20          | 1                   | 2.6  | na        | 40                    | 57        |
|                               |  | AEW    | 1.3 to 1.4/g    | 20               | 5                   | 2.6  | na        | 40                    | 57        |
|                               |  | AEW    | 2.7 to 3.0/g    | 50               | 1                   | 2.6  | na        | 40                    | 57        |
|                               |  | AEW    | 4.0/g           | 50               | 5                   | 2.6  | na        | 40                    | 57        |
|                               | Lactic acid bacteria   | na     | 1.9/g           | na               | 5                   | na   | na        | 3.6                   | 81        |
|                               | <i>Listeria monocytogenes</i>                                      | AEW    | 2.8/leaf        | 22               | 3                   | 2.5  | 1130      | 45                    | 86        |
|                               |  | NEW    | 4.0/g           | 30               | 5                   | 7    | >750      | 22 to 198             | 109       |
|                               |  | NEW    | 2.5/ml          | 25               | 10                  | 6.5  | 850       | 50                    | 32        |
|                               | Psychotrophs   | na     | 3.3/g           | na               | 5                   | na   | na        | 3.6                   | 81        |
| <i>Salmonella</i> Typhimurium | NEW  | 2.5/g  | 30              | 5                | 7                   | >750 | 22 to 198 | 109                   |           |
|                               | NEW  | 2.9/ml | 25              | 10               | 6.5                 | 850  | 50        | 32                    |           |
| <i>Staphylococcus aureus</i>  | NEW  | 2.8/ml | 25              | 10               | 6.5                 | 850  | 50        | 32                    |           |
| Potatoes (diced)              | Aerobic bacteria   | NEW    | 0.1/g           | 23               | 4                   | 6.8  | na        | 20                    | 39        |
| Radish (shreds)               | Aerobic bacteria   | NEW    | 0.5/g           | 23               | 3                   | 6.8  | na        | 20                    | 39        |

TABLE 4. *Continued*

| Food products    | Microorganisms                  | EW                  | Reduction (log)     | Temperature (°C) | Exposure time (min) | pH  | ORP (mV) | Active chlorine (ppm) | Reference |
|------------------|---------------------------------|---------------------|---------------------|------------------|---------------------|-----|----------|-----------------------|-----------|
| Spinach (leaves) | Aerobic bacteria                | NEW                 | 2.3/g               | 23               | 3                   | 6.8 | na       | 20                    | 39        |
|                  | <i>Enterococcus faecalis</i>    | NEW                 | 3.5/ml              | 25               | 10                  | 6.5 | 850      | 50                    | 32        |
|                  | <i>Escherichia coli</i>         | NEW                 | 2.6/ml              | 25               | 10                  | 6.5 | 850      | 50                    | 32        |
|                  | <i>Listeria monocytogenes</i>   | NEW                 | >4.9/ml             | 25               | 10                  | 6.5 | 850      | 50                    | 32        |
|                  | <i>Salmonella</i> Typhimurium   | NEW                 | 2.3/ml              | 25               | 10                  | 6.5 | 850      | 50                    | 32        |
|                  | <i>Staphylococcus aureus</i>    | NEW                 | >4.3/ml             | 25               | 10                  | 6.5 | 850      | 50                    | 32        |
| Strawberries     | Aerobic bacteria                | AEW                 | 1.6/strawberry      | na               | 10                  | 2.6 | 1130     | 32.1                  | 56        |
|                  | Coliforms                       | AEW                 | 2.4/strawberry      | na               | 10                  | 2.6 | 1130     | 32.1                  | 56        |
|                  | Fungi                           | AEW                 | 1.6/strawberry      | na               | 10                  | 2.6 | 1130     | 32.1                  | 56        |
| Tomatoes         | <i>Escherichia coli</i>         | NEW                 | 5.0/cm <sup>2</sup> | 23               | 1                   | 8.2 | 745      | 93                    | 20        |
|                  | <i>Escherichia coli</i> O157:H7 | AEW                 | 7.6/tomato          | 23               | na                  | 2.6 | 1140     | 30                    | 11        |
|                  |                                 | NEW                 | 4.9/cm <sup>2</sup> | 23               | 1                   | 8.2 | 745      | 93                    | 20        |
|                  | <i>Listeria monocytogenes</i>   | AEW                 | 7.5/tomato          | 23               | na                  | 2.6 | 1140     | 30                    | 11        |
|                  |                                 | NEW                 | 4.7/cm <sup>2</sup> | 23               | 1                   | 8.2 | 745      | 93                    | 20        |
|                  | <i>Salmonella</i> Enteritidis   | AEW                 | 7.4/tomato          | 23               | na                  | 2.6 | 1140     | 30                    | 11        |
| NEW              |                                 | 4.3/cm <sup>2</sup> | 23                  | 1                | 8.2                 | 745 | 93       | 20                    |           |

<sup>a</sup> na, not available; <sup>b</sup> *Salmonella* Typhimurium and *Salmonella* Enteritidis

TABLE 5. Antimicrobial activity of AEW and NEW on various food products

| Food products                                     | Microorganisms                  | EW  | Reduction (log)            | Temperature (°C) | Exposure time (min) | pH  | ORP (mV) | Active chlorine (ppm) | Reference |
|---|---------------------------------|-----|----------------------------|------------------|---------------------|-----|----------|-----------------------|-----------|
| <b>Fish and seafood:</b>                          |                                 |     |                            |                  |                     |     |          |                       |           |
| Carp (skin)                                       | Aerobic bacteria                | AEW | 2.8/cm <sup>2</sup>        | 25               | 15                  | 2.2 | 1137     | 41                    | 72        |
| Carp (filets)                                     | Aerobic bacteria                | AEW | 2.0/g                      | 25               | 15                  | 2.2 | 1137     | 41                    | 72        |
| Oysters   | <i>Vibrio parahaemolyticus</i>  | AEW | 1.1/g                      | na <sup>a</sup>  | 240                 | 2.8 | 1131     | 30                    | 90        |
|   | <i>Vibrio vulnificus</i>        | AEW | 1.1/g                      | na               | 240                 | 2.8 | 1131     | 30                    | 90        |
| Tilapia (skin)                                    | <i>Escherichia coli</i> O157:H7 | AEW | 0.6 to 0.8/cm <sup>2</sup> | 23               | 1 to 10             | 2.5 | 1159     | 120                   | 37        |
|   | <i>Vibrio parahaemolyticus</i>  | AEW | 2.6/cm <sup>2</sup>        | 23               | 10                  | 2.5 | 1159     | 120                   | 37        |
| Tuna (filets)                                     | Aerobic bacteria                | AEW | 1.0/g                      | na               | na                  | na  | na       | 50                    | 38        |
|   | Aerobic bacteria                | AEW | 1.0/g                      | na               | na                  | na  | na       | na                    | 111       |
| Salmon (filets)                                   | <i>Escherichia coli</i> O157:H7 | AEW | 0.5/g                      | 22               | 2                   | 2.6 | 1150     | 76-90                 | 83        |
|   |                                 | AEW | 1.1/g                      | 35               | 64                  | 2.6 | 1150     | 76-90                 | 83        |
|   | <i>Listeria monocytogenes</i>   | AEW | 0.4/g                      | 22               | 2                   | 2.6 | 1150     | 76-90                 | 83        |
| <b>Carcasses, raw meat and ready-to-eat meat:</b> |                                 |     |                            |                  |                     |     |          |                       |           |
| Chicken carcasses                                 | Aerobic bacteria                | AEW | 1.3/ml rinse               | 4                | 45                  | 2.6 | 1150     | 50                    | 27        |
|   | <i>Campylobacter jejuni</i>     | AEW | 2.3/g                      | na               | 40                  | 2.5 | 1140     | 47                    | 44        |
|   | Coliforms                       | AEW | 1.1/ml rinse               | 4                | 45                  | 2.6 | 1150     | 50                    | 27        |
|   | <i>Escherichia coli</i>         | AEW | 1.1/ml rinse               | 4                | 45                  | 2.6 | 1150     | 50                    | 27        |
|   | <i>Salmonella</i> Typhimurium   | AEW | 0.8/ml rinse               | 4                | 45                  | 2.6 | 1150     | 50                    | 27        |
| Chicken wings                                     | <i>Campylobacter jejuni</i>     | AEW | 3.0/g                      | 4 or 23          | 10 or 23            | 2.6 | 1082     | 51.6                  | 84        |
| Frankfurters, ham                                 | <i>Listeria monocytogenes</i>   | AEW | <1.0/g                     | 25               | 0.3                 | 2.3 | 1130     | 36                    | 26        |
| Frankfurters                                      | <i>Listeria monocytogenes</i>   | AEW | 1.5/g                      | 25               | 15                  | 2.3 | 1130     | 36                    | 26        |

TABLE 5. *Continued*

| Food products      | Microorganisms                | EW  | Reduction (log)     | Temperature (°C) | Exposure time (min) | pH  | ORP (mV) | Active chlorine (ppm) | Reference |
|--------------------|-------------------------------|-----|---------------------|------------------|---------------------|-----|----------|-----------------------|-----------|
| Pork               | Aerobic bacteria              | AEW | 1.2/cm <sup>2</sup> | na               | 0.3                 | 2.8 | 1144     | 68                    | 25        |
|                    | <i>Campylobacter coli</i>     | AEW | 1.8/cm <sup>2</sup> | na               | 0.3                 | 2.8 | 1144     | 68                    | 25        |
|                    | Coliforms                     | AEW | 1.2/cm <sup>2</sup> | na               | 0.3                 | 2.8 | 1144     | 68                    | 25        |
|                    | <i>Escherichia coli</i>       | AEW | 1.1/cm <sup>2</sup> | na               | 0.3                 | 2.8 | 1144     | 68                    | 25        |
|                    | <i>Listeria monocytogenes</i> | AEW | 1.2/cm <sup>2</sup> | na               | 0.3                 | 2.8 | 1144     | 68                    | 25        |
|                    | <i>Salmonella</i> Typhimurium | AEW | 1.7/cm <sup>2</sup> | na               | 0.3                 | 2.8 | 1144     | 68                    | 25        |
| <b>Shell eggs:</b> |                               |     |                     |                  |                     |     |          |                       |           |
|                    | <i>Escherichia coli</i>       | AEW | 4 to 6/egg          | na               | hourly 0.3          | 2.1 | 1150     | 8                     | 92        |
|                    | <i>Listeria monocytogenes</i> | AEW | 3.7/egg             | na               | 5                   | 2.7 | 1089     | 16                    | 88        |
|                    |                               | AEW | 1 to 4/egg          | na               | hourly 0.3          | 2.1 | 1150     | 8                     | 92        |
|                    | <i>Salmonella</i> Enteritidis | AEW | 2.3/egg             | na               | 5                   | 2.7 | 1089     | 16                    | 88        |
|                    | <i>Salmonella</i> Typhimurium | AEW | 4 to 6/egg          | na               | hourly 0.3          | 2.1 | 1150     | 8                     | 92        |
|                    | <i>Staphylococcus aureus</i>  | AEW | 3 to 6/egg          | na               | hourly 0.3          | 2.1 | 1150     | 8                     | 92        |

<sup>a</sup> na, not available

TABLE 6. Antimicrobial activity of sequential BEW and AEW treatment on various food products

| Product           | Microorganisms   | Reduction (log)     | Temperature (°C)     | Exposure time (min)  | pH                    | ORP (mV)               | Active chlorine (ppm) | Reference |
|-------------------|--|---------------------|----------------------|----------------------|-----------------------|------------------------|-----------------------|-----------|
| Chicken carcasses | Aerobic bacteria   | 2.4/ml rinsate      | BEW: na <sup>a</sup> | na                   | BEW: 11.6             | BEW: -795              | BEW: 0                | 27        |
|                   | Coliforms  | 1.6/ml rinsate      | AEW: 4               |                      | AEW: 2.6              | AEW: 1150              | AEW: 50               |           |
|                   | <i>Escherichia coli</i>  | 1.5/ml rinsate      |                      |                      |                       |                        |                       |           |
|                   | <i>Salmonella</i> Typhimurium                                      | 2.1/ml rinsate      |                      |                      |                       |                        |                       |           |
| Carp (skin)       | Aerobic bacteria   | 2.6/cm <sup>2</sup> | BEW: 25<br>AEW: 25   | BEW: 15<br>AEW: 15   | BEW: 11.6<br>AEW: 2.2 | BEW: -885<br>AEW: 1137 | BEW: 0.9<br>AEW: 41   | 72        |
| Cucumbers         | Aerobic bacteria   | 2.0/cucumber        | na                   | BEW: 5               | BEW: 11.3             | BEW: -870              | BEW: na               | 56        |
|                   | Coliformes   | 1.7/cucumber        |                      | AEW: 5               | AEW: 2.6              | AEW: 1130              | AEW: 32               |           |
|                   | Fungi  | 2.0/cucumber        |                      |                      |                       |                        |                       |           |
| Frankfurters      | <i>Listeria monocytogenes</i>                                      | <1.0/g              | BEW: 25<br>AEW: 25   | BEW: 0.3<br>AEW: 0.3 | BEW: na<br>AEW: 2.3   | BEW: na<br>AEW: 1130   | BEW: na<br>AEW: 36    | 26        |
| Lettuce           | Aerobic bacteria   | 2.0/g               | na                   | BEW: 1<br>AEW: 1     | BEW: 11.4<br>AEW: 2.6 | BEW: -870<br>AEW: 1140 | BEW: na<br>AEW: 30    | 55        |
|                   | <i>Escherichia coli</i> O157:H7 and <i>Salmonella</i> <sup>b</sup> | 1.8/g               | BEW: 20<br>AEW: 20   | BEW: 5<br>AEW: 5     | BEW: 11.4<br>AEW: 2.6 | na                     | BEW: 0<br>AEW: 40     | 57        |
|                   |  | 2.7/g               | BEW: 50<br>AEW: 4    | BEW: 1<br>AEW: 1/5   | BEW: 11.4<br>AEW: 2.6 | na                     | BEW: 0<br>AEW: 40     | 57        |
|                   |  | 4.0/g               | BEW: 50<br>AEW: 4    | BEW: 5<br>AEW: 1/5   | BEW: 11.4<br>AEW: 2.6 | na                     | BEW: 0<br>AEW: 40     | 57        |
| Shell eggs        | <i>Listeria monocytogenes</i>                                      | 3.0/egg             | na                   | BEW: 1               | BEW: 11.2             | BEW: -940              | BEW: 0                | 88        |
|                   | <i>Salmonella</i> Enteritidis                                      | 3.7/egg             | na                   | AEW: 1               | AEW: 2.7              | AEW: 1089              | AEW: 16               |           |
| Strawberries      | Aerobic bacteria   | 1.0/strawberry      | na                   | BEW: 5               | BEW: 11.3             | BEW: -870              | BEW: na               | 56        |
|                   | Coliformes   | 2.4/strawberry      |                      | AEW: 5               | AEW: 2.6              | AEW: 1130              | AEW: 32               |           |
|                   | Fungi  | 1.0/strawberry      |                      |                      |                       |                        |                       |           |

<sup>a</sup> na, not available; <sup>b</sup> *Salmonella* Typhimurium and *Salmonella* Enteritidis



