A TRIAL TO SUSTAIN THE SAFETY OF CHICKEN FILLET USING NEUTRAL ELECTROLYZED WATER (NEW)

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ABSTRACT

Electrolyzed water (EW) is considered as a novel broad-spectrum and high-performance bactericide that has gained immense popularity over the last few years. EW offers several advantages over other sanitizers for sanitation of food, contact and noncontact surfaces, such as safety, effective disinfection and easy operation, relatively inexpensive and environmentally friendly. The present study investigates the properties of neutral electrolyzed water (NEW) using NaCl solution with three concentrations (1%, 12% and 25%) against the most common pathogenic three food borne microorganisms (E. coli O157:H7, Salmonella enteritidis and Staphylococcus aureus) individually, in which fresh chicken breast samples were subjected to artificial infection with those microorganisms (10¹⁰CFU/ml) then followed by immersion separately in Neutral Electrolyzed water (NEW) with the three concentrations (1%, 12% and 25%) for 1,5 and ten minutes at room temperature. Reduction percentage was estimated for evaluating their antimicrobial ability. The results revealed high reduction percentage of the three food borne microorganisms for ten minutes at concentration of 25%, Also concentration 12% revealed high reduction percentage for *E. coli* O157:H7 and *S. Enteritidis* only at 1,5 and ten minutes. While the lowest reduction percentage that was estimated at concentration 1% for one minute against S. aureus by 72.344 \pm 1.265. The present study demonstrated that (NEW) was very effective in reducing and/or eliminating the food-borne bacterial contamination.

Keywords:

Neutral electrolyzed water (NEW), food-borne microbial contamination, *E. coli* O:157H7, S. *Enteritidis*, *S. aureus*, antimicrobial disinfectants.

INTRODUCTION

Staphylococcus aureus is a major pathogen in food industry and listed among the top 5pathogens causing food-borne illness (Sun *et al.*, 2012). Salmonella and Staphylococcus aureus are the top from five germs causing food poisoning in the United States (Rahman *et al.*, 2016). Salmonella and ECHC were estimated to be the leading cause and should beresponsible for 30% of food-borne illness hospitalization in United States individually (Jadeja and Hung 2014). Also Enterohemorrhagic Escherichia coli (EHEC) and salmonella have been reported as the causal factor of food-borne illness outbreak (Switaj *et al.*, 2015 and Ebel *et al.*, 2016).

Food safety is of crucial importance. However, each year, 48 million people become sick in the United States from one of 250 identified food borne diseases, 128,000 are hospitalized, and 3000 die (**Xiaoting** and **Jiangang 2019**).

Electrolyzed water (EW) as a novel cleaning and inactivation technology is generated in an environmentally friendly method from NaCl and distilled water. It is potentially applicable to no thermal food and processing. Its remarkable advantages include the environment friendly type, which poses no threat to humans after used, the ability for on-site generation, which avoids the chlorination problems during transport, storage and handling (Hricova et al., 2008). EW is generally recognized as safe (GRAS) and already regarded as a legitimate food additive in USA, Japan, and Korea. It is becoming more attractive because of its easy production and low-cost materials, chlorine off-gassing and noncorrosive to equipment (Len et al., 2000; Jadeja and Hung 2014 and Xuan et al., 2017). EW is produced by electrolysis of NaCl solution, which is the only chemical material. It has fewer adverse effects on human health and the environment owing to its chemical composition and nearneutral pH (Kim et al., 2000 and Ding et al., 2015a). The salt concentration and electrolysis time have positive correlations with the free chlorine concentration, which might be explained by considering that, the electrolysis efficacy of the electrolysis cell and the separation efficacy of the ion exchange membrane are greatly decreased with increasing flow rate and salt concentration (Kiura *et al.*,2002).

EW is considered as an effective disinfectant in food decontamination and preservation. Its disinfection efficacy against different food borne pathogens, e.g., *L. monocytogenes*, *E. coli* O157:H7, *S. aureus*, *S. typhimurium*, and *V. parahaemolyticus* have been investigated

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(McCarthy and Burkhardt 2012; Wang *et al.*, 2014a, b and Al-Holy and Rasco 2015). Subrota *et al.* (2012) generated EO water by electrolysis of sodium chloride in a cell containing inert positively and negatively charged platinum electrodes separated by a bipolar membrane. A salt solution as 12% NaCl and subjecting the electrodes to direct current voltage. Two types of water are produced simultaneously. EO water, with low pH (2.3 - 2.7), high oxidation-reduction potential (ORP, >1000 mV), high dissolved oxygen and contains free chlorine (concentration depends on the EO water machine setting), is produced from anode side (an electrolyzed acidic solution). However, electrolyzed reduced (ER) water, with high pH (10.0 - 11.5), high dissolved hydrogen, and low ORP (800 to 900 mV), is produced from the cathode side (an electrolyzed basic aqueous solution).



Fig.(1): Schematic diagram of EO water generation system (Subrota et al., 2012).

A study was done to investigate the properties of electrolyzed oxidizing (EO) water for the inactivation of pathogen and to evaluate the chemically modified solutions possessing properties similar to EO water in killing *Escherichia coli* O157:H7.A five-strain cocktail (10^{10} CFU/ml) of *E. coli* O157:H7 were subjected to deionized water (control), EO water with 10 mg/liter residual chlorine, EO water with 56 mg/liter residual chlorine, the properties of EO water could be simulated by chemically-modifying deionized water with acetic acid and

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chlorine water. EO water and chemically modified deionized water are possessing similar properties of EO water and were effective in inactivation of *E. coli* O157:H7 within 30 seconds treatment-time (**Kim** *et al.*, **2000**).

HOCl will change to Ocl in alkaline pH, whereas it will dissociate to Cl₂ at low pH values. The pH of EW determines the relative fractions of chlorine species in the solution (**Park** *et al.*, **2004**). **Jeong** *et al.* (**2007**) added that NEW has broad-spectrum inactivation ability with nonselective properties, which circumvents the growth of bacterial resistance and no negative influence on the sensory and quality of food by the using of acidic electrolyzed water (AEW), alkaline electrolyzed water (AIEW) and slightly acidic electrolyzed water (SAEW). The strongest chlorine form is HOCl, which has an inactivation efficacy that is 80-fold greater than that of OCl in an equivalent concentration when its pH range is from 5.0 to 6.5 (**Cao** *et al.*,**2009**).**Rahman** *et al.*(**2012**) suggested that an increase in the current(1.15-1.45 A) results in an increase in the pH,ACC, and ORP,which eventually enhances the antimicrobial ability toward *E. coli O157:H7* and*L.monocytogenes*.Inaddition,thereisa positive correlation between the conductivity of EW and the salt/acid concentration in the electrolyte solution. Chlorine compound is one of the most important factors responsible for the inactivation efficacy of EW.Moreover, a few reports have attributed the inactivation action to the ORP of EW (**Hao** *et al.*, **2012**).

High ORP of EW results in the destruction of layers of bacteria, disturbing the metabolic pathways and oxidation of sulfhydryl mixtures of cells. The result could accelerate the inactivation of bacterial cells. Therefore, the basic properties of EW including the available chlorine concentration(ACC) (Cl₂, $^{-}$ OCl and HOCl), pH and oxidization reduction potential (ORP), directly influence its sanitizing efficacy, whereas various electrolytic parameters such as the current, flow rate, salt concentration, electrolyte, electrode materials, water temperature, hardness, and storage environments have been reported to directly affect the propertiesofEW(Liaoet al., 2007;Ding et al., 2016andTkhawkhoet al., 2017).

Loss of chlorine by lighting is not significant during storage. In addition, a lower storage temperature (4 °C) made these basic properties of EW more stable than that stored at 25 °C and maintained its bactericidal efficiency over 12 months (Nagamatsu *et al.*, 2002; Fabrizio and Cutter 2003 and Robinson *et al.*, 2012).

Xuan et al. (2016) stated that a closed-dark container was a more conducive condition for

EW storage changes in basic properties (pH, ORP, and ACC) during storage. Furthermore, different types of EW showed different storage characteristics. **Rahman** *et al.*, (2012) determined the changes in ACC of low-concentration electrolyzed water (LcSAEW, 10 mg/L, pH of 6.8 -7.4) under closed and open conditions. They reported that the ACC of LcSAEW is gradually decreased from 10 to 0 mg/L in 7 days under the open-dark condition compared to 10 - 0 mg/L in 21 days under the closed-dark condition. The preheating method increased the ACC level of EW and enhanced its inactivation efficacy (Forghani *et al.*2015).

Neutral electrolyzed water, has near neutral pH value (6-8), giving similar antimicrobial mechanism but result in less metal surface corrosion or skin irritation as AEW (Huang *et al.*, 2008 and Cui *et al.*, 2009). Also NEW is more stable than AEW during the storage period (Nagamatsu *et al.*, 2002). Chuang *et al.*, (2013) stated that the membrane-less electrolysis container is more productive, stable, convenient and economic because of expendable ion-selective membrane is not utilized during electrolysis NEW can be produced by electrolyzing soft tap water with sodium chloride as the only chemical additive. The basic properties of NEW, available chlorine concentration (ACC), pH, and oxidization reduction potential (ORP) are vital factors on the basic properties of NEW and then influence its inactivation efficiency. Neutral electrolyzed water has been extensively used for inactivating food-borne bacteria. Different producing equipment and parameters greatly influence the properties of NEW during production period, preparation setting including current, water flow rate, salt/acid concentration, electrolyte and electrode, water temperature and hardness, storage environments and so on (Machado *et al.* 2016 and Zhang *et al.* 2016).

Hsu *et al.* (2019) demonstrated that, the membrane-less electrolyzed water (MLEW) with 30 minutes of electrolyzing process to 850 mL of concentrated solution NaCl (6.15 M) (357.4 g NaCl in 1Liter water), FAC concentration of NaCl solution would rise up to over 10,000 mg/L. and compared with two commercially available chlorine-related antimicrobial agents including bleach and chlorine dioxide (ClO2) usually used in the food-processing factory, to evaluate antimicrobial effects against food-borne related microorganisms, including Enterohemorrhagic *Escherichia coli*.(ECHC), Salmonella spp. and *Staph. aureus* individually and demonstrated that MLEW is very effective in reducing the food-borne microbial contamination.



Fig. (2): The schematic diagram of hand-made membrane-less electrolyzing device. (Hsu et al., 2019). When EO water comes into contact with organic matter, or is diluted by tap water or reverse osmosis (RO) water, it becomes ordinary water again. Thus, it's less adverse impact on the environment as well as users' health. Moreover, compared with other conventional disinfecting techniques,EO water reduces cleaning times, is easy to handle, has very few side effects, and is relative cheap as well as (Tanaka et al., 1999). Chemicals used for cleaning and disinfection are expensive and represent an operating expense for the dairy producer. Once the initial capital investment is made to purchase an EO water generator, the only operating expenses are water, salts and electricity to run the unit (Walker et al., 2005).

MATERIAL AND METHODS

This work chose the neutral electrolyzed water (NEW) [as the membrane-less electrolyzed water (MLEW)] for its antimicrobial agent against the most common pathogenic three foodborne illness microorganisms (*E.coli* O_{157} , *Salmonella Enteritdis* and *S. aureus*).

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Preparation of Membrane-less electrolyzed water (MLEW).

The NEW used in this study was generated by hand-made membrane-less electrolyzing device according to **Chuang** *et al.* (2013) and Hsu *et al.* (2019).The device consists of 850 mL cylinder polycarbonate (PP) container (height: 15 cm; diameter: 10.5 cm) filled with NaCl solution with different concentration (1%, 12% and 25%) separately. Two Pt/Ti base electrodes module (10×2 cm2) was installed inside the PP container as cathode and anode with the gap of 0.8 cm between electrodes with thirty minutes of electrolyzing process, was applied giving 9±2 A electrical current inputs.Obtained NEW were labeled and stored in glass closed containers at refrigerator temperature (4°C).

The pH value of the NEW solution was measured using pH meter (Julle C8 Sensory combination Phelectrod Garden Grove (CA92841).

Bacterial Culture:

Cultural bacterial population for each tested microorganism was determined according to **FDA (2001)** by tenfold serial dilution of 0.1 mL aliquot on EMB, XLD and Baird parker media for *E. coli* O157:H7, *Salmonella Enteritidis* and *S. aureus*, respectively. These strains were obtained from Reference Laboratory For Food Safety, Animal Health Research Institute (AHRI), Dokki, Egypt. The final bacterial population of the tested suspensions was adjusted to 10¹⁰ colony forming unit CFU/mL for subsequent experiments.

Rapid antimicrobial evaluation of NEW in vitro.

For rapid evaluation of the neutral electrolyzed water (NEW) using NaCl solution with the three concentrations (1%, 12% and 25%) for its antimicrobial agent against the most three selected food-borne microorganisms (*E.coli* O_{157} , *Salmonella Enteritdis* and *S. aureus*), were determined by well diffusion method and disc infusion. according to Clinical Laboratory Standards (**CLSI**, 2001) on EMB, XLD and Baird parker media, respectively.

Challenge trials:

Each10 g of fresh chicken breast samples (9 samples for each microorganism) were subjected to dipping in the bacterial population of the tested suspensions adjusted to 10^{10} colony forming unit CFU/mL to each microorganism separately and was left in refrigerator for 30 min for attachment, after that they were subjected to immersion in different NaCl concentrations (1%, 12%, and 25%) for three different time (1min, 5 min and 10 min) at room temperature with control positive and negative samples.

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The experiment was repeated triple using NaCl solution with three concentrations (1%, 12% and 25%) against the most three food borne pathogenic microorganisms (*E. coli* O157:H7, *Salmonella Enteritidis* and *S. aureus*).

All experimental values showed the means of three different experiments with 3 replicates of the inactivating treatment per experiment. The Statistical Product and Service Solutions (SPSS version 20) program was utilized for statistical evaluation of the obtained data.

Significant differences between inactivating tests with respect to bacterial reduction were analyzed by one-way ANOVA at a significance level of 0.05.



Fig. (3): Represented samples for one microorganism against NEW in three different NaCl concentrations (1%, 12% and 25%) for different time (1, 5 and 10 minutes) with control positive and negative samples.

RESULTS AND DISCUSSION

To improve the microbial safety of chicken meat, various techniques have been used for the reduction of bacterial contaminants (González-Fandos and Dominguez, 2007 and Hyeon *et al.*, 2013).

In recent years, an increasing number of publications have shown that EW hurdle technology is considered as a potential food decontamination process, which can improve the microbial quality and safety and extend the shelf life of fresh product. For instance, electrolyzed water, could be used for acceptable food safety, the recent trend in the food industry is to maintain an improved quality of food without compromising food safety.

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Regulations limited the amount of free accessible chlorine (FAC) in solutions used in food contact applications to below 20 ppm. a level at which it is ineffective, and partly due to its noticeable chlorine smell. There is a need for a food-safe, non-tainting composition with reduced associated cost implications and/or environmental implications.

The effect of EW on the inactivation of microorganisms is greatly influenced by a number of factors such as pH, ORP, flow rate of electrolytes, temperature, etc. The killing mechanism of EW can be attributed to the active chlorine species including Cl₂, HOCl, and ⁻OCl. As apart from active chlorine species, others such as reactive oxygen species (ROS) also contribute to the killing of microorganisms. The most active form of EW is HOCl, where HOCl attacks the cell wall, cell membrane, DNA, mitochondria and enzymes of microbial cells, which leads to cell death. However, there is little effect of ROS and ⁻OCl on microbial cells. The high oxidation reduction potential (ORP) of EW could remarkably influence the EW disinfection activity by allowing penetration of the outer and inner membranes. In addition to ORP, a reduced pH also significantly influences the disinfection power of EW (Liao *et al.*,2007).

NaCl Concentration	pН
1%	8.340±0.045
12%	7.780±0.147
25%	7.290±0.127

Table (1): pH value in the three NEW concentrations (1%, 12% and 25%).

Results are presented as means ± standard error of the three trials: PH values versus different NEW concentrations.

In the present experiment, pH ranged from neutral at salt concentration of 25% (7.290 \pm 0.127) to slightly alkaline at salt concentration of 1 % (8.340 \pm 0.045). The distribution of fractions of FAC compounds in electrolyzed water is dependent on pH values and affects biochemical characteristics. Due to the single-cell chamber, neutralization occurs when hydroxide ions (OH⁻) from the negative pole contact with protons (H⁺) from the positive pole and then neutral electrolyzed water (NEW)with pHof 7-8 and an ORP of 750-900 mV is produced. (**Huang et al.,2008**) **and Cui et al.(2009**) have been approved that neutral electrolyzed water, has near neutral pH value (6-8), giving similar antimicrobial mechanism

but results in less metal surface corrosion or skin irritation as AEW. In addition, slightly acidic electrolyzed water (SAEW) with a pH of 5.0-6.5 and an ORP of 800-900 mV is produced by electrolysis of HCl or in combination with NaCl in a EW generation equipment using an electrolysis chamber without the separating membrane (**Forghani** *et al.*, **2015**).

Table (2): E. coli O157:H7 reduction % on chicken breast samples against NEW of three different NaCl concentrations (1%, 12% and 25%) for different time (1, 5 and 10 minutes) with control group 4x108cfu/ml.

NaCl Concentration	Control group	1min.	5min.	10 min.
1%		91.365 ±0.694 ^b	92.841 ±0.750 ^b	99.518 ±0.291 ^a
12%	4x10 ⁸ cfu/ml	99.461 ±0.236 ^a	99.75±0.239 ^a	99.568 ±0.288 ^a
25%		99.441 ±0.238 ^a	99.737±0.338 ^a	99.987±0.123 ^a

Results are presented as means ± standard error of the three trials.

The values with different superscript letters in a row are significantly different (p<0.05).

E. coli O157:H7 contamination reduction with NEW treated chicken breast samples.

When NEW with different concentration (1%, 12% and 25%) was applied on chicken breast samples against *E. coli* O157:H7, antimicrobial effect was represented by nearly complete reduction when subjected for 10 minute with the three concentrations with no significant difference. Concentration 1% for 1 min. and 5 min showed reduction percent of 91% and 92% respectively, this may be need to subsequent treatment as rapid chilling, freezing or another treatment .While EW immersion solutions of concentration 12% and 25% showed no significance difference at different treated time (1,5 and 10 minutes).

This results was agree with **Hsu** *et al.*,(**2019**) **and Kim** *et al.*, (**2000**) as they showing that inactivating efficiency of NEW against ECHC in the test suspension , totally inactivated by NEW with FAC of 100 mg and 50 mg/L after 10 second and 30 second treatment, respectively. Also, revealed that NEW performed rapid and effective antimicrobial reaction against ECHC in the short time contacts and expected that longer contact time between antimicrobial agent and bacterial bring better inactivating effect.



Fig. (4): E. coli O157:H7 on EMB agar and its reduction (a) by well diffusion method where 0.1ml of control sterilized distilled water (X), NEW 1% (X1) and NEW 12% (X2) and (b) its reduction by NEW 1% (Y),12% (Y1)and 25% (Y2) NaCl concentrations using disc infusion.

Table (3):S.Enteritids reduction % on chicken breast samples against NEW by three different NaCl concentrations (1%, 12% and 25%) for different time (1, 5 and 10 minutes) with control group 6x10⁸cfu/ml.

NaClConcentration	Control group	1min.	5min.	10 min.
1%	6x10 ⁸ cfu/ml.	93.960 ± 0.695^{b}	96.499 ± 0.311^{b}	99.634 ± 0.181^{a}
12%		98.326 ± 0.247^{a}	99.747 ± 0.093^{a}	99.827 ± 0.086^{a}
25%		99.505 ± 0.206^{a}	99.925 ± 0.056^{a}	99.993 ± 0.089^{a}

Results are presented as means \pm standard error of the three trials. The values with different super script letters in a row are significantly different (p<0.05).



Fig. (5):(a): Salmonella Enteritidis on XLD agar and(b) Reduction by NEW 1% and 12% NaCl concentrations using disc infusion.

S.Enteritids contamination reduction with NEW treated chicken breast samples.

The result in this study indicates that NEW was better effective antimicrobial agents against *Salmonella Enteritidis* 10 min. for the three concentrations (1%, 12% and 25%) which represented by 99.634 \pm 0.181, 99.827 \pm 0.086 and 99.993 \pm 0.089, respectively with no significant difference. Also treatment with NEW 12% and 25% for 1 min and 5 min resulted inacceptable inactivating efficiency without significance difference. While rapid treatment for 1 min to 1% showing reduction % by 93.960 \pm 0.695 and might needed further hurdle technology. It can be expected that longer contact time between NEW as antimicrobial agent and bacteria and/or increase salt concentration bring better inactivating effect.

The result in this study was agree with **Hsu** *et al.*,(**2019**) as reported that NEW are better effective antimicrobial agents against *Salmomnella spp*. while rapid treatment is needed.

Table (4): Staph. aureus reduction % on chicken breast samples against NEW by three different NaCl concentrations (1%, 12% and 25%) for different time (1, 5 and 10 minutes) with control group 2.2x10⁸ cfu/ml.

NaCl Concentration	control group	1min.	5min.	10 min.
1%		$72.344 \pm 1.265^{\circ}$	90.420 ± 1.280^{b}	94.584 ± 0.446^{b}
12%	2.2x10 ⁸ cfu/ml.	94.343 ± 0.791^{b}	99.303 ± 0.286^{a}	99.547 ± 0.385^{a}
25%		97.806 ± 0.618^{a}	99.410 ± 0.287^{a}	99.856 ± 0.068^{a}

Results are presented as means ± standard error of the three trials.

The values with different superscript letters in a row are significantly different (p<0.05).



Fig (6): (a): *S. aureus* on Baird parker agar and (b) reduction by NEW 1% and 12% NaCl concentrations using disc infusion.

Staph.aureus contamination reduction with NEW treated chicken breast samples.

Application of NEW immersion solutions by 25% concentration and dipping for 1, 5 and, 10 minutes shown to be more efficient in terms of microbial reduction, as well as. Concentration of 12% for 5 minutes and 10 minutes showed no significant difference and could serve as a

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promising alternative during the processing of poultry meat samples to decrease food-borne pathogens risk. When EW was applied alone to study the mechanism of action in *S. aureus*, the results indicated that EW caused leakage of intracellular potassium, 2, 3, 5-triphenyl tetrazolium chloride-dehydrogenase activity inhibition and ultra-structure disruption of the cell (**Ding** *et al.*, **2016**).

There was no complete elimination of the pathogens in this study on treatment by 1% concentration for 1 minute (72.344 \pm 1.265%).By elongate treatment time to 5 and 10 minutes reduction percent reached to 90.420 \pm 1.280 and 94.584 \pm 0.446 for the same concentration, respectively.Use of hurdle technology to enhance the antimicrobial activity of EW is needed to the concentration 1% for the time 1,5,10 minutes and to the concentration of 12% for 1 minute and this has already been reported by a number of researchers as **Luo and Oh** (**2016**) who demonstrated that, however the individual sanitization efficacy of EW was found to be insufficient to completely inactivate or decontaminate many food products. Also **Hsu** *et al.*, (**2019**) reported that experiments against *S. aureus*, NaOCl did not present significant inactivating efficiency no matter 10 second or 30 second treatment (survival rate were 57% and 17%). NEW presented < 10 CFU/mL survival rate against *S. aureus* contamination if longer contact time available.

In the food industry, the novel approach of hurdle technology has been introduced to guarantee microbial safety, nutritional quality, and the economic viability of food products. Hurdle technology, also known as combination preservation, combined methods, barrier technology, combined processes, and combination techniques, is the application of two or more basic food preservation techniques to reduce the extreme conditions of individual treatments and enhance their effectiveness (**Khan** *et al.*, **2017**). Hurdle technology provides safe, stable, and improved nutritional quality. Though an individual food preservation treatment, for instance, electrolyzed water, could be used for acceptable food safety. The recent trend in the food industry is to maintain an improved quality of food without compromising food safety (**Rostami** *et al.*, **2016**).

There is a need for a food-safe, non-tainting disinfecting composition which can be used within the food industry to disinfect food processing lines and equipment and can be used during and/or between foods processing provides improved anti-microbial efficacy and

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requires a shorter and/or less expensive cleaning cycle. Also it is important to maintain quality variables of the meat samples such as color, texture, and sensory characteristics which showed no significant differences between the treated with NEW and the untreated control one in this study.

As for the safety of EW, there were many reports about the acute oral toxicity test the skin irritation test the acute eye stimulation test. No changes were observed in skin sensitization test, oral mucosal stimulation test, return mutation test, and chromosomal abnormality test. Rats were administered with sodium hypochlorite(500-2000 mg/kg)for 104weeks and sodium hypochlorite (500, 1000 mg/kg) was administered to rats for 103 weeks and carcinogenic. Together with these results and other toxicity tests and literature, the safety of EW under practical use conditions was considered to be satisfactory (**Morita** *et al.*, **2011**).

CONCLUSION

The present infestation relates to the use of NEW, was effective in reducing and/or eliminating food-borne pathogens such as *E. coli* O157:H7, Salmonella spp and *S. aureus* on food products.

It is an attractive and effective method for the food industry owing to its easy production, low-cost materials, high disinfection efficacy, and broad spectrum of disinfection activity, a dressed carcass is washed with tap water. Therefore, by using NEW in place of the tap water or a pretreatment of meat products with clean water and frequent change to NEW immersion solutions could be necessary. Also in the food industry, equipment such as for example processing lines and tools need to be disinfected in order to minimize the risk of microbial contamination. Microbial contamination can lead to spoilage of food products, reduced shelf life and/or food poisoning of the consumer. As a result, microbial contamination issues cost the food industry billions of pounds a year.

In the future, in the slaughter treatment using NEW in place of the tap water, it is considered that microbiological safety of meat can become increasingly and is expected to improve food safety in various food manufacturing domains.

REFERENCES

- Al-Holy M.A. and Rasco, B.A. (2015): The bactericidal activity of acidic electrolyzed oxidizing water against Escherichiacoli O157:H7, SalmonellaTyphimurium, and Listeria monocytogenes on raw fish, chicken and beef surfaces. Food control. 54:317–321.
- Cao, W.; Zhu, Z.W.; Shi, Z.X.; Wang, C.Y. and Li, B.M. (2009): Efficiency of slightly acidic electrolyzed water for inactivation of Salmonella Enteritidis and its contaminated shell eggs. International Journal of Food Microbiology, 130: 88-93.
- Chuang, C. Y.; Yang, S.; Huang, H. C.; Luo, C. H.; Fang, W.; Hung, P. C. and Chung, P. R. (2013): Applying the membrane-less electrolyzed water spraying for inactivating bio aerosols. AAQR, 13(1): 350-359.
- Clinical and Laboratory Standards Institute (CLSI) (2001): Performance Standards for Antimicrobial Disk Susceptibility Tests. Approved Standards, Wayne, PA. CLSI document M2-A7.
- Cui, X.D.; Shang, Y.C., Shi, Z.X., Xin, H. and Cao, W. (2009): Physicochemical properties and bactericidal efficiency of neutral and acidic electrolyzed water under different storage conditions. Journal of Food Engineering 91, 582 -586.
- Ding, T., Ge Z.; Shi, J.et al., (2015a): Impact of slightly acidic electrolyzed water (SAEW) and ultrasound on microbial loads and quality of fresh fruits.LWT-Food Sci Technol.,60:1195-1199.
- Ding, T.; Ge, Z; Shi, J.et al., (2015b): Impact of slightly acidic electrolyzed water (SAEW) and ultrasound on microbial loads and quality of fresh fruits. LWT-Food sci Technol 60 (2): 1195-1199.
- Ding, T.; Xuan, X-T, Li, J.et al.; (2016): Disinfection efficacy and mechanism of slightly acidic electrolyzed water on *Staphylococcus aureus* in pure culture. Food Control 60:505-510.
- Ding, T.; Xuan, X.T.; Liu, D.H.et al., (2015a): Electrolyzed water generated using a circulating reactor. Int J Food Eng. 11(1):79-84.
- Ebel, E. D.; Williams, M. S.; Cole, D.; Travis, C. C.; Klontz, K. C.; Golden, N. J. and Hoekstra, **R. M. (2016):** Comparing characteristics of sporadic and outbreak-associated foodborne illnesses, United States, 2004 -2011. - Emerg Infect Dis 22(7): 1193.
- Fabrizio, K. and Cutter, C. (2003): Stability of electrolyzed oxidizing water and its efficacy against cell suspensions of Salmonella Typhimurium and Listeria monocytogenes. J. Food Prot., 66 (8):1379-1384.
- **FDA**, (2001): Microbiological Methods and Bacteriological Analytical Manual (BAM).
- Forghani, F.; Park, J.H. and Oh, D.H. (2015): Effect of water hardness on the production and microbicidal efficacy of slightly acidic electrolyzed water. Food Microbiol 48:28-34.

440 j. Egypt. act. med. Assac 80, no 2. 425 - 444 / 2020/

- González-Fandos, E. and Dominguez, J. L. (2007): Effect of potassium sorbate washing on the growth of *Listeria monocytogenes* on fresh poultry. *Food Control*, 18: 842- 846.
- Hao, J.; Qiu. S.; Li, H.et al., (2012): Roles of hydroxyl radicals in electrolyzed oxidizing water (EOW) for the inactivation of *Escherichia coli*. Int. J. Food Microbiol.
- Hricova, D.; Stephan, R. and Zweifel, C. (2008): Electrolyzed water and its application in the food industry. J Food Protect 71(9):1934 -1937.
- Hsu, Y. F.1; Chuang, C. Y.; Huang, H. C.1 and Yang, S.1. (2019): Applying membrane-less electrolyzedwater forinactivating pathogenicmicroorganisms, applied ecology and environmental research, 17 (6):15019 -15027.
- Huang, Y.R.; Hung Y.C.; Hsu S.Y.et al., (2008): Application of electrolyzed water in the food industry. Food Control, 19 (4):329 -345.
- Hyeon, J. Y.; Chung, G. T.; Bing, S. H.; Kwon, K. S.; Lee, H. H.; Kim, S. J. et al. (2013): Afoodborne outbreak of *Staphylococcus aureus* associated with fried chicken in republic of Korea. J. *Microbiol. Biotechnol*, 23: 85 - 87.
- Jadeja, R. and Hung, Y.C. (2014): Efficacy of near neutral and alkaline pH electrolyzed oxidizing water to control *Eschericchia coli* O 157:H7 and *Salmonella Typhimurium* DT 104 from beef hides. Food Control, 41:17-20.
- Jeong J.; Kim, J.Y.; Cho M *et al.* (2007): Inactivation of *Escherichia coli* in the electrochemical disinfection process using a Pt anode. Chemosphere, 67(4):652 659.
- Khan, I.; Tango, C.N.: Miskeen, S.*et al.*, (2017): Hurdle technology: a novel approach for enhanced food quality and safety-a review. Food Control, 73:1426-1444
- Kim,C.;Hung Y.C. and Brackett, R.E.(2000): Roles of oxidation–reduction potential in electrolyzed oxidizing and chemically modified water for the inactivation of food-related pathogens. J Food Prot., 63 (1):19 -24.
- Kiura, H.; Sano K.;Morimatsu, S.*et al.*, (2002): Bactericidal activity of electrolyzed acid water from solution containing sodium chloride at low concentration, in comparison with that at high concentration. J. Microbiol Method, 49 (3):285-293.
- Len,S.V.;Hung,Y.C.;Erickson,M.and Kim,C.(2000):Ultravioletspectrophotometric characterization and bactericidal properties of electrolyzed oxidizing water as influenced by amperage and pH. Journal of Food Production, 63: 1534 -1537.
- Liao, L.B.; Chen W.M. and Xiao, X.M. (2007): The generation and inactivation mechanism of oxidation–reduction potential of electrolyzed oxidizing water. J Food Eng., 78:1326-1332.

j.Egypt.net.med.Assac 80, no 2, 425 - 444/2020/

- Luo, K., and Oh, D.H. (2016): Inactivation kinetics of *Listeria monocytogens* and *Salmonella enerica* serovar Typhemurium on fresh-cut bell pepper treated with slightly acidic electrolyzed water combined with ultrasound and mild heat. Food microbiology, 53,165-171.
- Machado, I..; Meireles, A.; Fulgêncio, R.; Mergulhão, F.; Simões, M. and Melo, L. F. (2016): Disinfection with neutral electrolyzed oxidizing water to reduce microbial load and to prevent biofilm regrowth in the processing of fresh cut vegetables. - Food Bioprod. Process, 333-340.
- McCarthy S. and Burkhardt, W. (2012): Efficacy of electrolyzed oxidizing water against *Listeria monocytogenes* and *Morganellamorganii* on conveyor belt and raw fish surfaces. Food Control, 24 (1):214-219.
- Morita. C.; Nishida, T. and Ito, K. (2011): Biological toxicity of acid electrolyzed functional water: effect of oral administration on mouse digestive tract and changes in body weight. Arch Oral Biol., 56 (4):359 -366.
- Nagamatsu, Y.; Chen K.K.; Tajima, K.*et al.*, (2002): Durability of bactericidal activity in electrolyzed neutral water by storage. Dent Mater J 21(2):93-104.
- Park, H.; Hung, Y.C. and Chung, D. (2004): Effects of chlorine and pH on efficacy of electrolyzed water for inactivating *Escherichia coli* O157:H7 and *Listeriamonocytogenes*. International Journal of Food Microbiology, 91:13-18.
- Rahman, S.M.E.; Khan, I. and Oh, D.H. (2016): Electrolyzed water as a novel sanitizer in the food industry: current trends and future perspectives. Compr Rev Food Sci Food Saf, 15:471-490.
- Rahman, S. M. E., J.; Park, K. B.; Song, N. A.; and Oh. D. H. (2012): Effects of slightly acidic low concentration electrolyzed water on microbiological, physicochemical, and sensory quality of fresh chicken breast meat. J. Food Sci., 77: 35-41. Len, S.V.
- Robinson, G.; Thorn, R. and Reynolds, D. (2012): The effect of long-term storage on the physiochemical and bactericidal properties of electrochemically activated solutions. Int J MolSci., 14 (1):457-469.
- Rostami, Z.; Ahmad, M.A.; Khan, M.U. *et al.* (2016): Food preservation by hurdle technology: a review of different hurdle and interaction with focus on foodstuffs. J Pure ApplMicrobiol, 10:2633-2639.
- Subrota,H.; SurajitMandal,P.S.Minz,ShilpaVij,YogeshKhetra,B.P.Singh,Dipika Yadav(2012): Electrolyzed Oxidized Water (EOW) Non-Thermal Approach for Decontamination of Food Borne Microorganisms in Food Industry, *Food and Nutrition Sciences*, 3:760-768.
- Sun, J.L.; Zhang, S.K.; Chen, J.Y.et al., (2012): Efficacy of acidic and basic electrolyzed water in eradicating *Staphylococcus aureus* biofilm. Can J Microbiol, 58 (4):448 - 454.

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j.Egypt.net.med.Assoc 80, no 2. 425 - 444 /2020/

- Switaj, T. L.; Winter, K.J. and Christensen, S. R. (2015): Diagnosis and Management of Foodborne Illness. - Am FAM Physician, 92(5): 358-365.
- Tanaka, N.; Fujisawa, T. and Daimon, T. (1999): "The Effect of Electrolyzed Strong Acid Aqueous Solution on Hemodi- alysis Equipment," *Artificial Organs*. 23, (12):1055 -1062.
- Tkhawkho, L.; Jackson, K; Nitzan, O. *et al.*, (2017): Destruction of clostridium difficile spores colitis using acidic electrolyzed water. Am J Infect Control 45 (1):1053.
- Walker, S. P.; Demirci, A. and Graves, R. E. (2005): "CIP Cleaning of a Pipeline Milking System Using Electrolyzed Oxidizing Water," *International Journal of Dairy Technology*, 58: 65-73.
- Wang, J.J.; Sun, W, S.; Jin, M.T et al., (2014a): Fate of Vibrio parahaemolyticus on shrimp after acidic electrolyzed water treatment. Int J Food Microbiol 179:50 -56.
- Wang, J.J.; Zhang,Z.H.; Li, J. B. et al., (2014b): Modeling Vibrioparahaemolyticus inactivation by acidic electrolyzed water on cooked shrimp using response surface methodology. Food Control, 36 (1):273-279.
- Xiaoting, X.and Jiangang, L.(2019): Generation of Electrolyzed Water© Springer Nature Singapore Pte Ltd. and Zhejiang University Press, Hangzhou 2019
- Xuan, X.T.; Fan, Y.F; Ling, J.G.*et al.*, (2017): Preservation of squid by slightly acidic electrolyzed water ice. Food Control, 73:1483-14893.
- Xuan, X.T.; Wang, M.M.; Ahn J et al., (2016): Storage stability of slightly acidic electrolyzed water and circulating electrolyzed water and their property changes after application. J Food Sci., 81(3):E610–E617.
- Zhang, C.; Li, B.; Jadeja, R.; Fang, J. and Hung, Y. C. (2016): Effects of bacterial concentrations and centrifugations on susceptibility of Bacillus subtilis vegetative cells and *Escherichia coli* 0157: H7 to various electrolyzed oxidizing water treatments. – Food Control, 60: 440-446.

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الملخص العربى

دائما هناك حاجة إلى تركيبة آمنة وغير ملوثة للأغذية للإستخدام في صناعة الأغذية لتحسين الفعالية ضد مسببات الأمراض التي تنتقل عن طريق الأغذية والتي يمكن أن تحسن الجودة وتطيل العمر الإفتراضي للمنتجات الطازجة وتتطلب أقصر و / أو أقل تكلفة ويمكن إستخدامه لتطهير خطوط ومعدات تجهيز الأغذية في الموقع ويمكن إستخدامها أثناء و / أو بين معالجة الطعام بتعتبر المياه المتأينة كهربائيا (EW) مطهر جديد في السنوات الأخيرة. يحتاج إنتاج ماء EWإلى الماء والملح فقط (كلوريد الصوديوم). تتمتع مياه EW بالمزايا التالية مقارنة بعوامل التنظيف التقليدية الأخرى: التطهير الفعال والتشغيل السهل وغير مكلفة نسبيًا وصديقة للبيئة. وفي هذة التجربة تم تطبيق إستخدام المياه المتعادلة المؤكسدة كهربائيا بثلاث تركيزات مختلفة من ملح الطعام (كلوريد الصوديوم) (1% ، 12% ، 25%) على شرائح الدجاج الطازجة والتي تم عدواها منفردة بثلاث أهم ميكروبات مسببة للتسم الغذائي وهي الإيشريشاكولاي والسالمونيلا إنتريتيدس والميكروب العنقودي الذهبي بتركيز 10¹⁰خلية / مل ثم معالجتها بالغمس في الماء المتأين المتعادل على فترات (1، 5 ، 10 دقائق).وقد اوضحت النتائج نسب اختزال تمثلت في %0.123±99.987 للإيشريشاكولاي و %99.993±0.089 للسالمونيلا إنتريتيدس و %0.068 ± 99.856 للميكروب العنقودي الذهبي عندالتركيز الأعلى من الصوديوم كلوريد 25% لمدة 10 دقائق بينما الإختزال الجزئي أظهرته النتائج عند التعامل لمدة 1 دقيقة للتركيز 1% لميكروب العنقودي الذهبي.المياة المتأينة (EW) لها تأثير على تعطيل الكائنات الحية الدقيقة إلى حد كبير وتعزى آلية قتل EW إلى أنواع الكلور النشطة بما في ذلك Cl2، HOCl ، و -OCl ، أيضا تساهم أنواع أخرى مثل أنواع الأكسجين التفاعلية (ROS) في قتل الكائنات الحية الدقيقة ، بالاضافة الى الأس الهيدروجيني pH الذي يؤثر أيضًا بشكل كبير على قوة التطهير من EW.أكثر أشكال EW نشاط هو HOCl ، حيث يهاجم HOCl جدار الخلية وغشاء الخلية والحمض النووي والميتوكوندريا وإنزيمات الخلايا الميكروبية ، مما يؤدي إلى موت الخلية. أظهرت النتائج أن NEW لها نتائج واعدة في التحكم في نمو الميكروبات في الغذاء وتحسين مدة الصلاحية بالإضافة لتجنب التأثير السلبي للماء المتأين الحمضي (AEW)على الخصائص الحسية للمنتجات الغذائية.أصبحت سلاسل الغذاء معقدة في التعامل والتجهيز والنقل والتخزين ولذلك أصبح الإمداد الآمن بالأغذية مهمة صعبة مما يؤكد أن الماء المُحلُّل بالكهرباء يحتوي على الكثير من الإستخدامات المحتملة لصناعة المواد الغذائية كأحد التطبيقات الأساسية والمثبتة هو إستخدامه إستخدام فعال و تطبيقه مباشرة على المنتجات الغذائية الطازجة لتقليل عدد الكائنات الحية الدقيقة أو مسببات الأمراض الموجودة أوكمطهر على أسطح ملامسة الطعام ويعتبر هذا مفيد للصناعة لأنه ينطوي على الإنتاج في الموقع للمطهر و عدم وجود مواد كيميائية للتخزين أو التعامل مع تكاليف المواد الكيميائية للتعامل معها و يوفر العديد من المزايا مقارنة بالمطهرات الأخرى ، مثل السلامة والتطهير الفعال والتشغيل السهل وغير المكلف نسبيًا والصديق للبيئة.

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