



## Physicochemical Properties and Bactericidal Effects of Neutral Electrolyzed Water against *Escherichia coli* and *Salmonella enterica* on Ground Pork

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### ABSTRACT

This study evaluated physicochemical and antibacterial properties of neutral electrolyzed water (NEW) produced by electrolyzing NaCl solutions. pH, total chlorine content (TC) and oxidation-reduction potential (ORP) of NEW increased to equilibrium values when increasing NaCl concentration (0.20% - 1.5%) and electrolysis time (0 – 240 minute). The pH and ORP values increased sharply in the first 15-min of the electrolysis and then was stabilized in the ranges of 8.5-9.5 and 400-500 mV, respectively. Increasing NaCl concentration did not change the stabilized values of pH and ORP, but significantly increased ( $p < 0.05$ ) TC. Furthermore, we studied antibacterial activity of NEW against *Escherichia coli* and *Salmonella enterica* in suspension and in ground pork. Interestingly, 0.85% NaCl NEW after 10-min electrolysis reduced 7 log CFU/mL of *E. coli* and 2 log CFU/mL of *S. enterica*. This resistance of *S. enterica* toward NEW was possibly due to its biofilm-forming ability.

**Keywords:** Neutral electrolyzed water, *Escherichia coli*, Ground pork, *Salmonella enterica*, Sodium chloride.

### INTRODUCTION

Microbial foodborne intoxication is one of the major concerns in the food industry today. Electrolyzed water is a product with many potential applications, primarily in terms of eliminating microorganisms, for the food industry in many countries. EW is made by electrolysis of solutions of NaCl, MgCl<sub>2</sub>, KCl or HCl<sup>1</sup>. Depending on the electrolyte used for electrolysis, acidic, basic or

neutral electrolyzed water can be obtained. Acidic electrolyzed water has been used to treat different bacteria such as *Escherichia coli*, *Salmonella enteritidis*, *Salmonella typhimurium*, *Listeria monocytogenes*, etc. with an inactivation up to 6.0 log CFU/mL of bacteria. The main disadvantage of acidic neutralized water is the gradual evaporation of Cl<sub>2</sub> at low pH, resulting in a reduction of bactericidal ability over time. In addition, evaporated Cl<sub>2</sub> gas adversely affects human health and pollutes the



environment<sup>2</sup>. Moreover, low pH of electrolyzed water can cause equipment corrosion, and thus limiting its application range<sup>3</sup>.

Neutral electrolyzed water (NEW) can inactivate many microorganisms without affecting the sensory quality of food<sup>4,5</sup>. NEW is generated by electrolyzing solution of a chloride salt in a system without a diaphragm. In the anodic compartment, products such as  $H^+$ ,  $H_2O_2$ ,  $O_3$ ,  $O_2$ ,  $Cl_2$ ,  $HClO$ ,  $ClO_3^-$ , etc., are formed, while  $OH^-$ ,  $H_2$ ,  $NaOH$ , etc., are formed in the cathode compartment<sup>6</sup>. With careful control of the electrolysis conditions, NEW can be used for microbial treatment without deteriorating food quality<sup>7,8</sup>. The purpose of this study was to evaluate the effects of NaCl concentration on physicochemical properties of NEW and the ability to eliminate *Escherichia coli* and *Salmonella enterica*, two main pathogens causing food poisoning as well as common intestinal diseases in food. To the best of our knowledge, there was no study about using NEW to disinfect ground pork, which has large surface area and complex surface morphology.

## MATERIALS AND METHODS

### Materials

*Escherichia coli* (VTCC-B-482) and *Salmonella enterica* (VTCC-B-480) bacterial strains used in this study were purchased from the Institute of Microbiology and Biotechnology (Vietnam National University, Hanoi). The bacteria were cultured in Nutrient Broth medium with continuous shaking (250 rpm) at 37°C for 24 hours. Bacterial suspensions were diluted to  $10^8$ - $10^9$  CFU/mL (8-9 log CFU/mL) to be used in this study.

The meat used was fresh ground pork purchased in a local supermarket. The pork was tested to ensure that it complied with the EUR-Lex standard (88/657/EEC) about meat for direct human consumption or for industry (Table 1).

**Table 1: Microbiological criteria for fresh pork meat**

Criteria	Satisfactory
Total viable count, CFU/g	<10 <sup>5</sup> *
Coliform, CFU/g	<10 <sup>2</sup>
<i>E. coli</i> , CFU/g	<10 <sup>2</sup>
<i>Staphylococcus aureus</i> , CFU/g	<10 <sup>2</sup>
<i>Clostridium perfringens</i> , CFU/g	<10 <sup>2</sup>
<i>Salmonella</i> , CFU/25g	Not detected

\*<10<sup>6</sup>. for ground pork

### Production of Neutral Electrolyzed Water (NEW)

Sodium chloride solutions (0.2, 0.5, 0.85 and 1.5%, w/v) were sterilized at 121°C for 15 minutes. The sterilized solutions were cooled to room temperature and then poured into the electrolysis tank (Fig. 1). The electrodes were two titanium plates (10 cm × 10 cm) coated with mixed metal oxides, including  $RuO_2$ ,  $IrO_2$  and  $TiO_2$ , to prevent electrochemical corrosion. Electrolysis of the NaCl solutions was conducted with a controlled current of 3A and a voltage of 20 V using a DC power supply (CPS – 3205E, China).

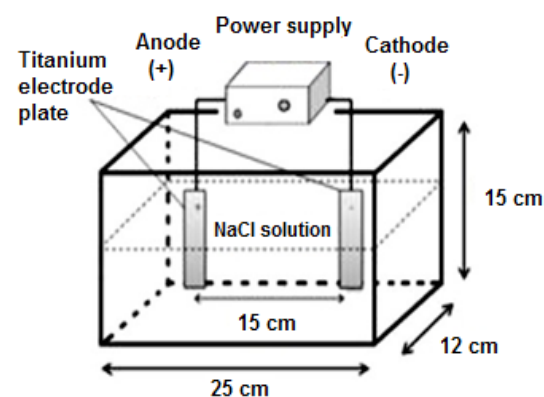


Fig. 1. Diaphragmless electrolysis tank of NaCl solutions

### Total chlorine

The pH and the oxidation-reduction potential of the electrolyzed water were measured in situ using a pH meter (SevenEasy, Switzerland) and an ORP tester (KKmoon Pen-Type ORP Meter, China)<sup>8</sup>. TC of the electrolyzed water was determined based on ISO 7393-3:1990 titration method<sup>9</sup>. Briefly, an aliquot of the electrolyzed water was mixed with 10.0 mL of a standardized 0.015 M  $Na_2S_2O_3$  solution. Afterwards, 1 g of KI, 2 mL of 0.87 M  $H_3PO_4$  solution and 1 mL of 0.5% (w/v) starch solution were successively added. The solution was then titrated with a standard  $KIO_3$  solution until the appearance of a blue colour, which remained for at least 30 s.

### Antibacterial activity of NEW against *E. coli* and *S. enterica* on Petri dishes

An *E. coli* or *S. enterica* suspension (0.1 mL) was mixed with NEW (0.9 mL) under stirring for 5 min at room temperature. To determine the concentration of survived bacteria, the mixture was then serially diluted by a 0.1% pept on solution and then spread on Nutrient Broth agar Petri dishes. The Petri dishes were subsequently kept at 37°C for 24 h before the number of formed colonies

on each dish was counted. The survived bacterial concentration was transformed to log CFU/mL of bacterial suspension. To conduct a negative control experiment, a phosphate buffer saline solution (0.9 mL, pH 9.0), instead of NEW, was mixed with the bacterial suspension. After 5 min of stirring, the concentration of survived bacteria was determined as aforementioned<sup>10</sup>.

#### Antibacterial activity of NEW against *E. coli* and *S. enterica* in contaminated pork

To contaminate the pork, fresh pork (25 g) was mixed thoroughly with 5 mL of a bacterial suspension of *E. coli* or *S. enterica* and then incubated at 37°C for 24 hours. The contaminated pork was then put in a sealed flask for 1 h at 4°C for stabilization. To test the antibacterial effect of NEW on the contaminated pork, peptone (1%, 100 mL) and NEW (900 mL) were added to the flask. The flask was then shaken continuously for 5 min at room temperature (~30°C). After that, the concentration of survived bacteria was determined as in the method above<sup>11,12</sup>. The negative control experiment was conducted in the same procedure with a phosphate buffer saline solution (0.9 mL, pH 9.0) replacing the neutral electrolysed water.

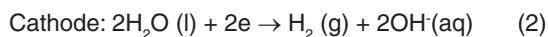
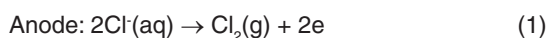
#### Statistical analysis

All the antibacterial experiments were carried out in triplicate. Statistical analyses, including ANOVA and Tukey's test ( $p < 0.05$ ) were conducted with SPSS statistical program package (version 20.0, USA).

## RESULTS AND DISCUSSION

#### Physicochemical properties of NEW during electrolysis

During diaphragmless electrolysis of NaCl solutions, the following main reactions take place<sup>8</sup>:



Under stirring conditions and without diaphragm:



The DC current transferred through the electrolyte solution resulted in the Ohmic heating effect that increased the temperature of the NaCl

solutions (Fig. 2). Moreover, the exothermic chemical reaction (3) was another reason for the temperature rise during electrolysis<sup>13</sup>. Higher concentrations of NaCl increased the electrical current and the extent of reaction (3), hence resulting in a higher temperature of the solution. However, the generated heat was exchanged with the environment, thus keeping a maximum temperature of about 45°C after 240 minute. From a practical point of view, it should be noted that high temperatures can lead to low solubility of chlorine gas, thus reducing TC values and the antibacterial effects of the NEW<sup>8,12</sup>. This implies the necessity of a cooling mechanism when using this electrolytic technology to produce neutral electrolysed water.

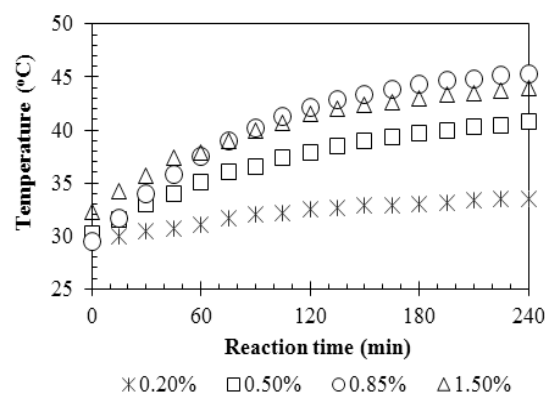


Fig. 2. Temperature of NaCl solutions during electrolysis

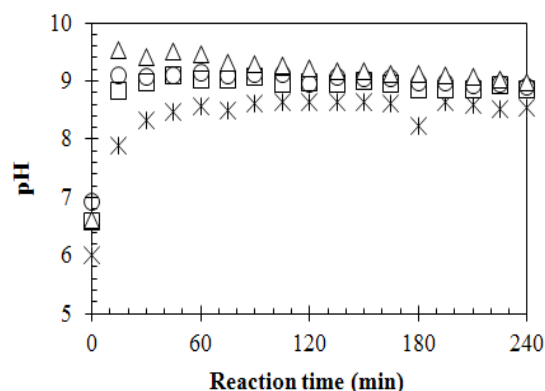


Fig. 3. pH of the NaCl solutions during electrolysis

Another important property of the electrolyzed water that can affect its point-of-use applicability is the pH. Too high or too low values of pH can result in corrosion and deteriorate the food quality. In the range of NaCl concentration from 0.2 to 1.5 % (w/v), the pH values of the electrolyte solutions increased rapidly and reached their equilibrium value (from 8.4 to 9.0) after approximately 60 min

of electrolysis (Fig. 3). The pH values were higher than 7.0 due to the presence of hypochlorite ions, which are weakly basic anions. These slightly basic pH values are acceptable in the food industry and can be considered neutral<sup>8</sup>. There are no significant differences between the pH values of the electrolyzed water with NaCl concentrations of 0.5% and higher<sup>13,14</sup>.

TC and ORP are important physicochemical properties of NEW that affect its antibacterial ability and technological applications. Their values should be high enough to ensure high antibacterial effects, but too high values of them can deteriorate the sensory quality of food and corrode equipment in contact<sup>8</sup>. Therefore, the values of TC, ORP and pH should be monitored and controlled during and after the electrolysis.

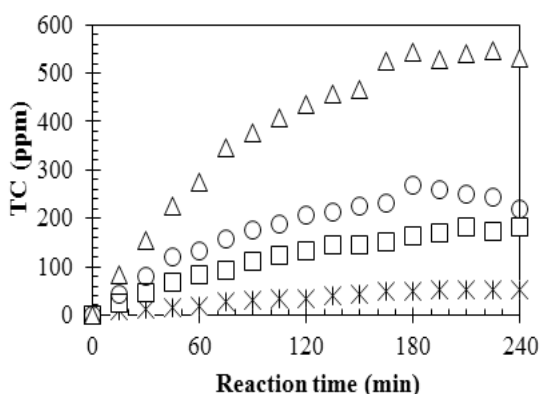


Fig. 4. Total chlorine (TC) in electrolyzed water during electrolysis

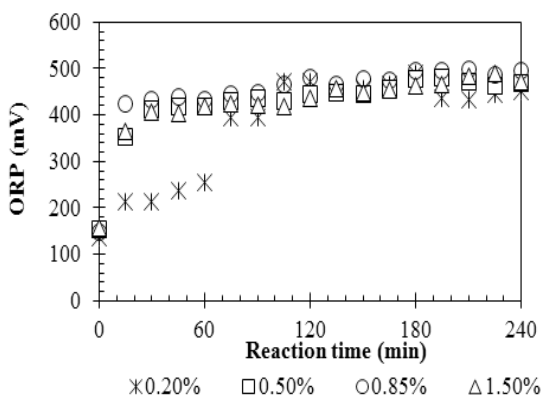


Fig. 5. Oxidation-reduction potential (ORP) of electrolyzed water during electrolysis

In general, NaCl concentration has a significant effect on properties of EW, especially

the total chlorine due to the formation of oxidizing agents ( $\text{ClO}^-$  and  $\text{Cl}_2$ ) in reactions (2) and (3)<sup>14,15,16</sup>. The TC increased gradually with electrolysis time and was proportional to NaCl concentration (Fig. 4). After 180 min, the TC values stopped increasing (1.5% NaCl) or even decreased (0.85% NaCl) due to the temperature increase in the NaCl solutions during electrolysis (Fig. 2), which caused partial evaporation of chlorine<sup>8</sup>.

Figure 5 shows that increasing the NaCl concentration from 0.2% to 0.5% resulted in a significant increase in the ORP during the first 60 minutes. However, no significant difference between the ORP values was observed at longer electrolysis times for all investigated NaCl concentrations. The chlorine compounds ( $\text{Cl}_2$  and  $\text{OCl}^-$ ) with high oxidizing ability are the reason for the increase in ORP values<sup>6,13</sup>. Previous studies showed that ORP and TC value are highly correlated with the ability to kill microorganisms of antiseptic solutions<sup>6,8,17</sup>.

#### Antibacterial activity of NEW against *E. coli* and *S. enterica* suspensions

In this study, the NEW from 0.85% NaCl solution was chosen to test the antibacterial effects because this concentration is close to that of commercial physiological saline. Fig. 6 demonstrates the high antibacterial effects of this NEW against *E. coli* in a suspension. After 10 min of electrolysis, the 0.85% NaCl solution containing approximately 30 ppm of TC reduced 9.23-log CFU/mL of *E. coli*. At the same time, *S. enterica* showed significant resistance toward NEW. Only 1.55-log CFU/mL reduction was observed for *S. enterica*, which corresponded to a 97% reduction in the bacterial concentration. Increasing electrolysis time to 20 min increased antibacterial effect to 2.42-log CFU/mL (99.6%) reduction of *S. enterica*.

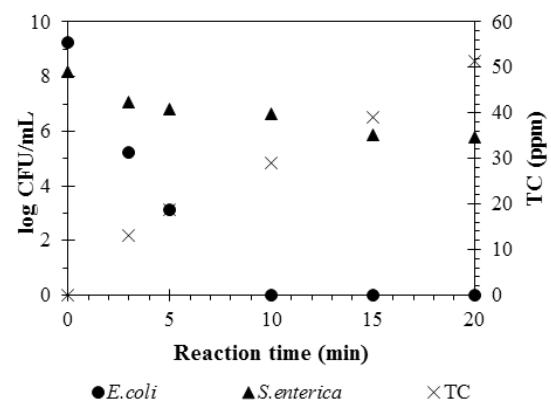


Fig. 6. Effect of NEW on the elimination of *E. coli* and *S. enterica* on NB agar

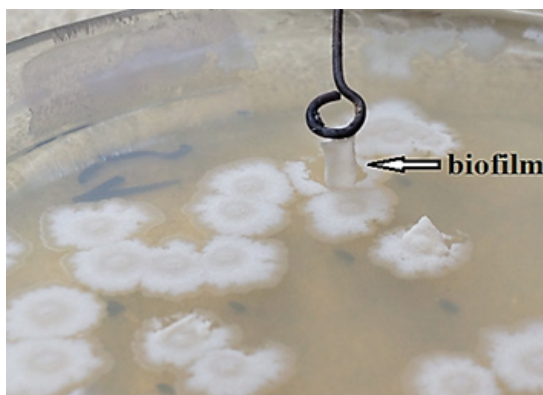


Fig. 7. Biofilm of *S. enterica* on NB agar

The pH and ORP values of NEW obtained from 0-20 min of electrolysis were not significantly different (Fig. 3 and 5). However, the TC value increased sharply during this period of electrolysis. Thus, we suppose that TC has a direct impact on the antibacterial effect of NEW. This result is in accordance with a previous study, in which hypochlorous acid and hypochlorite ions were the most active chlorine compounds in killing bacterial cells. The inactivation mechanism of these compounds was oxidation of sulfhydryl groups in important enzymes in the bacterial carbohydrate metabolism<sup>18</sup>.

Abadias<sup>19</sup> showed that NEW (48 ppm of TC) could reduce more than 5.4-log CFU/mL of *E. coli*

O157:H7 and *Salmonella choleraesuis*. Moreover, Issa-Zacharia<sup>11</sup> using slightly acidic and strong acidic electrolyzed waters (about 50 ppm of TC) reduced 5- to 6-log CFU/mL of *E. coli* and *Salmonella* ssp. Thus, the inactivation effectiveness of NEW in this study against *E. coli* was quite similar to previous studies. However, for *S. enterica*, the inhibition effects this study was quite lower than that of previous ones. A possible explanation was that the ability to create biofilm (Fig. 7) at high bacterial density has helped *S. enterica* to resist better the effects of EW than *E. coli*<sup>20</sup>. This biofilm, which is composed of polymer layers called glycocalyx, after forming can encapsulate the bacterial cells and create a protective surface that allows *Samonella* spp. to survive in harsh environments, including disinfecting and gene-modification agents<sup>21,22,23,24</sup>. Vestby<sup>21</sup> suggested that the bactericidal hypochlorite ions (OCl<sup>-</sup>) cannot get in contact with bacterial cell walls due to the blocking mechanism of organic layers in this biofilm.

#### Antibacterial activity of NEW against *E. coli* and *S. enterica* in contaminated pork

Ground meat is easy to be contaminated and hard to be disinfected due to its large surface area. However, NEW after treatment with NEW, the amount of *E. coli* and *S. enterica* as well as the aerobic microorganisms available on pork were significantly reduced (Table 2).

Table 2: Bacteridal activity of NEW at various electrolysis times against *E. coli* and *S. enterica* in contaminated pork

Electrolysis time (min)	TC (ppm)	Total viable count in contaminated pork (log CFU/g)	
		<i>E. coli</i>	<i>S. enterica</i>
0	0.00 ± 0.01a	7.94 ± 0.10a	7.54 ± 0.02a
3	13.12 ± 0.00b	6.65 ± 0.20b	6.60 ± 0.07b
5	18.63 ± 0.01c	6.08 ± 0.06c	5.82 ± 0.09c
10	29.14 ± 0.11d	ND	5.80 ± 0.15c
15	38.80 ± 0.20e	ND	5.78 ± 0.21c
20	51.00 ± 0.59f	ND	5.77 ± 0.16c

Results are expressed as mean ± standard deviation (n = 3). Different letters a, b, c in the same column indicate significantly different means (p<0.05). ND - Not detected

Figure 6 and Table 2 show that NEW after 10 min of electrolysis can completely eliminate *E. coli*, both in suspensions and in contaminated ground pork. The antibacterial effects of NEW on ground pork was lower than that in suspension, because the ground pork with complex surface

morphology acts as a physical barrier inhibiting the bactericidal effects of NEW<sup>25</sup>. In addition, Mahmoud<sup>26</sup> observed only 2-log CFU/g reduction in total bacteria on muscle meat after washing with acidic electrolyzed water (AEW) with 0.9% NaCl after 15 min of electrolysis. The difference in the

antibacterial effects in the study of Mahmoud<sup>26</sup> with that in our study (7.94-log CFU/g reduction) may be due to the difference in properties of acidic and neutral electrolyzed waters: ORP = 1137 mV, pH 2.2, 40.8 ppm TC chlorine in Mahmoud study<sup>26</sup> and those in the present study ORP = 425 mV, pH: 9.09, 29.14 ppm TC. Although AEW solution with higher ORP can have better higher antibacterial effects, but some studies showed this effect decreased markedly with EW with pH lower than 2.73,<sup>27</sup>. This can be explained from a chemical point of view: an acidic pH shift to equilibrium (3) to the left side, hence producing Cl<sub>2</sub>(g) in the form of tiny bubbles. The bubbles can escape easily from solution and has less contact areas with the bacteria cells than the soluble form of ClO<sup>-</sup>.

In this study, NEW after 10 min of electrolysis (29.14 ppm of TC) can completely inactivate 7.94-log CFU/g of *E. coli* on the ground pork sample. Because the maximum allowable level of *E. coli* contamination in fresh meat was 10<sup>2</sup> CFU/g and 10<sup>6</sup> for aerobic bacteria (according to Council Directive 88/657/EEC), NEW that can reduce from 5- to 6-log CFU/g can be used in the food industry<sup>16</sup>.

NEW after 20 min of electrolysis reduced only 1.77-log CFU/g of *S. enterica* (Table 2). Therefore, the treatment of *S. enterica* and bacteria available on pork meat by NEW has limited applicability. As mentioned above, the difference in results between the treatments of *E. coli* and *S. enterica* was due to the ability of *S. enterica* to create biofilms<sup>28</sup>. *Salmonella* is a Gram-negative bacterial family with a thick cell membrane<sup>29</sup> and a biofilm mechanism that envelops even neighbour cells to resist antimicrobial agents<sup>28</sup>. According to Habimana<sup>30</sup> and Corcoran<sup>31</sup>, when other aerobic bacteria are present together with *S. enterica* in the environment, especially *Pseudomonas*, *Staphylococcus* and *Pantoea*, etc., they promote *S. enterica* to create denser biofilms that enveloped the other bacteria, hence protect them from disinfecting agents. Therefore, it could be

the reason for reducing NEWs antibacterial ability against *S. enterica* and other bacteria present on the contaminated pork.

Although the results in the laboratory show that NEW did not completely inactivate 7.54-log CFU/g of *S. enterica* in contaminated pork, the NEW still can find applications in sterilizing meat in the food industry. In reality, no consumable food is allowed to be contaminated with Salmonella at such a high density. In addition, biofilms from *Salmonella* form only when bacterial density is higher than 10<sup>5</sup> CFU/g<sup>32</sup>. Therefore, it is feasible to apply NEW in the disinfection of *S. enterica* in food, agricultural and medical fields.

## CONCLUSION

This study showed that NEW can be used to disinfect meat contaminated aerobic bacteria, such as *E. coli* and *S. enterica*. Increasing NaCl concentration and/or electrolysis time increases physicochemical properties (temperature, pH, ORP, TC) and antibacterial activity of NEW. NEW from 0.85% NaCl solution and 10 min of electrolysis showed applicable results in disinfecting ability on ground meat. *S. enterica* at high density showed relatively high resistance toward NEW. This study suggests further investigations on the applicabilities of NEW on food contaminated with *S. enterica* at various extents.

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## Conflict of Interest

The authors declare no conflict of interest regarding the publication of this article.

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