Impact of low-concentrated acidic electrolysed water obtained by membrane electrolysis on the decontamination of meat microbiota

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Abstract

The influence of acidic electrolysed water (AEW) treatment on inactivation of pure bacterial cultures inoculated onto the surface of agarised media and surface microbiota of pork meat were examined. Low-concentrated AEW (low concentration of sodium chloride and low current electrolysis) was generated by electrolysis (5 or 10 min) of 0.001% or 0.01% NaCl solution. The number of viable microorganisms was determined using a plate count method. The effect of AEW on bacterial cell morphology were investigated using scanning electron microscopy (SEM). After treatment with AEW, a significant, about 3.00 log reduction of Pseudomonas fluorescens, Yersinia enterocolitica, Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Listeria monocytogenes, and Micrococcus luteus populations was observed. In the AEW treatment of pork, the highest reduction of total number of microorganisms (2.1 log reduction), yeast and moulds (2.5-2.6 log reduction), and psychrotrophs (more than 1 log reduction) was observed after spraying with 0.001% NaCl subjected to 10 min electrolysis. SEM revealed disruption and lysis of E. coli and S. aureus cells treated with AEW, suggesting a bactericidal effect. Higher available chlorine concentration (0.37-8.45 mg/L), redox potential (863.1-1049.8 mV), and lower pH (2.73-3.70) had an influence on the shape of bacteria and the number of breaks in the bacterial membrane.

Keywords: meat, bacterial cultures, acidic electrolysed water, antibacterial activity, decontamination.

Introduction

Food animals, infected or asymptomatic carriers, are sources of spoilage and pathogenic microorganisms. Animal integument, as well as animal faeces and the environment they live in may be a source of contamination for carcasses during the slaughtering, chilling, and cutting processes; meat products during processing, storage and handling; water, and other foods through contaminated manure; or may lead to direct transfer of pathogens causing infections of humans (26). Many processes have been proposed in order to eliminate or significantly reduce bacterial populations during food production. Organic acids, chlorine and chlorine derivates, hydrogen peroxide, and ozone are usually used in these processes (33). However, most of these methods were found absolutely unacceptable due to chemical residues, discoloration, high cost, or limited effectiveness. The development of effective sanitisers to decrease or eliminate microbial populations on food is an ongoing subject of interest (2). A decrease in foodborne diseases through reduction of pathogen contamination levels in meat products could reduce annual economic losses in medical costs, lost productivity, recalls, legal fees, and loss of businesses by $12 billion in the United States alone (26). Despite the availability of these treatment techniques and potential effectiveness of these interventions, researchers are investigating the use of other novel, antimicrobial agents to reduce or inhibit pathogenic or spoilage organisms related to fresh meat, and to do it more effectively and economically. Many techniques have been devised to decrease microbial contaminants on animal carcasses and on raw meat. The treatment with acidic electrolysed water (AEW) seems to be a promising method. AEW (also known as
electrolysed oxidising water or ionised water) is a potential alternative with environmentally friendly broad-spectrum microbial decontamination. AEW is a product of membrane electrolysis of a dilute sodium chloride solution in water ionisers where anode and cathode electrodes are separated by a membrane. AEW is obtained from the anode side and is characterised by low pH (<3), high oxidation-reduction potential (ORP > 1000mV), high dissolved oxygen and free chlorine content (2). It was tested and used as a disinfectant in the food industry and in other applications. It was reported that this kind of water is very effective in inactivation of Escherichia coli O157:H7 (29, 19, 23), Campylobacter jejuni (27), Salmonella enteritidis (8), and Listeria monocytogenes (28, 7). Electrolysed water is more effective, less dangerous and less expensive than most traditional disinfection methods (13). The major advantages of the AEW for inactivation of bacteria are less adverse environmental impacts and lack of difficulties with transportation and storage of potentially hazardous chemicals (14). Conversely, the disadvantages of AEW treatment are: reduced effectiveness due to the presence of protein (4) and production of a pungent chlorine gas, which causes discomfort for the operator (1). The article presents a way to reduce these disadvantages through the use of low-concentrated AEW.

This study had a dual purpose: firstly, to evaluate the efficacy of low-concentrated (low concentration of sodium chloride and low current of electrolysis) AEW in inactivating E. coli, S. aureus, L. monocytogenes, B. subtilis, M. luteus, P. fluorescens, Y. enterococitica with regard to its potential application in antimicrobial treatment of raw meat and meat contact surfaces; and secondly, to investigate the bactericidal effects of the water on cellular structures of selected bacteria.

Material and Methods

Bacterial cultures. The following test strains were used for testing AEW solutions: P. fluorescens (PCM 1994), Y. enterococitica (PCM 2080), E. coli (PCM 2560), S. aureus (PCM 2602), B. subtilis (PCM 2021), L. monocytogenes (PCM 2606), and M. luteus (PCM 1994). The bacterial strains were obtained from the culture collections of the Institute of Immunology and Experimental Therapy (Polish Academy of Sciences in Wroclaw). The strains were selected because they are bacterial species generally encountered in meat spoilage (5).

Preparation of electrolysed water solutions. AEW solutions were generated by membrane electrolysis of diluted salt solutions in various time spans (5 and 10 min) using a water ioniser (own design batch type generator, equipped with two titanium electrodes coated with 0.6 um layer of platinum). The current and voltage were set at 3 A and 300 V, respectively. Two variables: addition of 0.001% and 0.01% sodium chloride solutions (N) and electrolysis time (5 and 10 min) (E) were investigated. After electrolysis, AEW was collected from the anode side of the water generator. Freshly prepared AEW was examined. Non-electrolysed sodium chloride solutions at concentrations of 0.001% and 0.01% and deionised water were used as controls.

Analytical measurements. The pH and oxidation-reduction potential (ORP) of all types of water were measured with pH/ORP meter (Seven Multi™ model S40 Mettler Toledo) using a pH electrode (Inlab Routine Pro) and an ORP electrode (Inlab Redox Pro) respectively. Available chlorine concentration (ACC) was determined by the iodometric method (3).

Treatment of pure cultures. The microorganisms, except P. fluorescens and L. monocytogenes, were cultured for 18 h at 37°C. P. fluorescens was cultured at 25°C and L. monocytogenes was cultured in enrichment broth (BTL, Poland) or in BH broth (Merck, Poland). Bacterial optical density was measured in spectrophotometer Ray Leigh UV 18000 at 550 nm. Inoculum containing 10^7 CFU·mL⁻¹ was diluted (1:10, 1:100), and then each volume of each bacterial suspension was mixed with nine volumes of electrolysed and non-electrolysed salt solutions and incubated for 10 min. After 10-min treatment, 1 mL of the obtained solution was transferred to triplicate nutrient agar plates. Antibacterial activity was performed using the viable plate count method. Plates were incubated at 37°C for 24 h for each tested strain (except P. fluorescens: 25°C for 48 h). The results, which are expressed as log reduction, were calculated as shown in following formula (Eq.1) (31):

\[
\text{Log reduction} = \log_{10} \frac{A}{B}
\]

where:

A – number of viable microorganisms before treatment, 
B – number of viable microorganisms after treatment.

Sanitising treatment of pork meat and microbiological analysis. The research material was pork muscle (Longissimus thoracis) originating from the Dworecki Meat Plant (Golejowo, Poland). The material was collected 48 h after slaughter. Electrolysed sodium chloride solutions were prepared as previously described for the treatment of pure cultures. The 50-mm-thick muscle slices were sprayed with electrolysed and non-electrolysed sodium chloride solution for 60 s (immediately after solutions preparation). Sterile cotton swabs were used to collect samples from meat surface in accordance to ISO 18593:2004 (15). The template with the opening size of 5 cm × 4 cm was used to sample the same surface. The swab was placed in a test tube with a sterile saline solution (10⁻³) and further dilutions were prepared. The dilutions were prepared according to ISO 17604:2003 (17). Total number of microorganisms, psychrotrophs, yeast, and moulds were determined using plate method described in ISO 2293:1988 (14), ISO 17410:2001 (16)
and ISO 21527-1:2008 (18) respectively. Culture medium for the determination of total number of microorganisms was prepared using yeast extract (Merck, Poland), glucose (BTL, Poland), tryptone (BTL, Łódź, Poland), and agar (Merck, Poland). Colonies were counted after 72-h incubation at 30°C. Psychrotrophs were incubated at 6°C for 10 d on culture medium containing yeast extract, glucose, and enzyme-hydrolysed casein (BTL, Poland) and agar. Culture medium of yeast and moulds was prepared using yeast extract, glucose, agar and chloramphenicol (Sigma-Aldrich, Poland). Yeast and mould colonies were counted after 5-d incubation at 25°C. There were three replicate dilutions of each treatment. CFU·mL⁻¹ of each sample was calculated according to the following formula (Eq.2) (14):

\[
L = \frac{\Sigma C}{(n_1 + 0.1n_2)d^2/2}
\]

where:
- \( L \) - number of colonies per millilitre of the product (CFU·mL⁻¹),
- \( \Sigma C \) - sum of all colonies counted on all plates,
- \( n_1 \) - number of plates counted on all plates,
- \( n_2 \) - number of plates in the lower dilution,
- \( d \) - dilution corresponding to the first count (n₁).

The data were calculated using the following formula (Eq. 3) (15) and expressed as log₁₀ CFU/cm²,

\[
N_2 = \frac{N \cdot F}{A} / 31/
\]

where:
- \( N \) - count of microorganisms (CFU·mL⁻¹),
- \( A \) - test surface, 20 cm²,
- \( F \) - volume of diluent in test-tube (mL).

**Electron microscopy.** The effect of AEW on cell morphology was investigated using the E. coli and S. aureus strains. The 18-h E. coli (PCM 2560) and S. aureus (PCM 2602) cultures were centrifuged at 5000 \( \times \) g for 20 min. The appropriate amount of AEW (9 mL of AEW/0.1 g of bacteria) was added to the centrifuged inoculum of the tested bacteria. After 10 min of treatment with AEW, the samples were centrifuged again (5000 \( \times \) g for 10 min). The aqueous solution was decanted and the samples were immersed in 5 mL of 2.5 % glutaraldehyde to fix bacterial cells. The structure of bacterial cells was then analysed under scanning microscope as described by Kalifiski et al. (20).

**Statistical analysis.** The data were analysed using a 2-way ANOVA (Statistica, version 10 (StatSoft Poland). The effect of two independent categorical variables, namely concentration of NaCl and electrolysis time, was evaluated. Duncan’s multiple-range test was used to compare the differences among treatment means (P ≤ 0.05). The ACC data were analysed using the Kruskal-Wallis test followed by Mann-Whitney U test.

**Results**

The values of pH, redox potential, and available chlorine concentration of electrolysed sodium chloride solutions and its references used for treatment are presented in Table 1.

<table>
<thead>
<tr>
<th>Test designation*</th>
<th>Concentration of NaCl (N) (%)</th>
<th>Electrolysis time (E) (min)</th>
<th>pH</th>
<th>ORP (mV)</th>
<th>ACC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOE0</td>
<td>0</td>
<td>0</td>
<td>6.68 ± 0.02</td>
<td>460.08 ± 8.8</td>
<td>0° ± 0°</td>
</tr>
<tr>
<td>NOE5</td>
<td>5</td>
<td>5.25 ± 0.03</td>
<td>6.33 ± 0.03</td>
<td>433.06 ± 3.3</td>
<td>0° ± 0°</td>
</tr>
<tr>
<td>NOE10</td>
<td>10</td>
<td>6.17 ± 0.02</td>
<td>514.64 ± 8.5</td>
<td>0° ± 0°</td>
<td></td>
</tr>
<tr>
<td>NOE0.01E0</td>
<td>0</td>
<td>6.23 ± 0.06</td>
<td>479.14 ± 13.6</td>
<td>0° ± 0°</td>
<td></td>
</tr>
<tr>
<td>NOE0.01E5</td>
<td>0.001</td>
<td>3.70 ± 0.04</td>
<td>863.1 ± 4.3</td>
<td>0.37 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>NOE0.01E10</td>
<td>0.001</td>
<td>3.56 ± 0.04</td>
<td>957.1 ± 26.2</td>
<td>0.74 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>NOE0.01E0</td>
<td>0</td>
<td>6.00 ± 0.04</td>
<td>457.2 ± 3.3</td>
<td>0° ± 0°</td>
<td></td>
</tr>
<tr>
<td>NOE0.01E5</td>
<td>0.01</td>
<td>2.92 ± 0.06</td>
<td>1029.5 ± 1.5</td>
<td>4.12 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>NOE0.01E10</td>
<td>10</td>
<td>2.73 ± 0.02</td>
<td>1049.8 ± 4.4</td>
<td>8.45 ± 0.05</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Physicochemical properties of electrolysed and non-electrolysed sodium chloride solutions

<table>
<thead>
<tr>
<th>Test designation*</th>
<th>Log reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. fluorescens</td>
<td>Y. enterocolitica</td>
</tr>
<tr>
<td>NOE0</td>
<td>0.00 ± 0.02</td>
</tr>
<tr>
<td>NOE5</td>
<td>0.00 ± 0.02</td>
</tr>
<tr>
<td>NOE10</td>
<td>0.00 ± 0.03</td>
</tr>
<tr>
<td>NOE0.01E0</td>
<td>0.00 ± 0.03</td>
</tr>
<tr>
<td>NOE0.01E5</td>
<td>2.97 ± 0.08</td>
</tr>
<tr>
<td>NOE0.01E10</td>
<td>2.97 ± 0.04</td>
</tr>
<tr>
<td>NOE0.01E0</td>
<td>0.00 ± 0.04</td>
</tr>
<tr>
<td>NOE0.01E5</td>
<td>2.97 ± 0.07</td>
</tr>
<tr>
<td>NOE0.01E10</td>
<td>2.97 ± 0.05</td>
</tr>
</tbody>
</table>

Table 2. Inactivation of different bacteria by electrolysed and non-electrolysed salt solutions

a-b – means with different superscript letters in the same column are significantly different (P ≤ 0.05) according to the ANOVA.
Fig. 1a-i. Scanning electron micrographs of *E. coli* treated with electrolysed solutions: b) N0E5, c) N0E10, e) N0.001E5, f) N0.001E10, h) N0.01E5, i) N0.01E10 and non-electrolysed solutions a) N0E0, d) N0.001E0, g) N0.01E0

Fig. 2. Scanning electron micrographs of *S. aureus* treated with: a) non-electrolysed salt solutions (N0.01E0) and b) electrolysed water (N0.01E10)
Fig. 3. Changes in surface microflora on fresh meat after treatment of acidic electrolysed water solutions. Values with different letters (a–c) within the same concentration differ significantly (P < 0.05)

a) Total number of microorganisms

b) Yeast and moulds

c) Psychrotrophs
The pH of non-electrolysed sodium chloride solutions and deionised water (control) was near-neutral and its ORP was significantly lower than that of the electrolysed solutions tested. No free chlorine was detectable in deionised water. The highest ORP value (1049.8 ± 4.4) and free chlorine concentration (8.45 ± 0.05) was registered for 0.01% sodium chloride after 10 min of electrolysis. In comparison to the non-electrolysed salt solutions, ORP value was 2 fold greater. Increasing electrolysis time of NaCl solutions from 5 to 10 min, significantly affected redox potential, pH, and active chlorine concentration (Table 1).

Table 2 reports the results of the determination of the bactericidal activity of AEW. All seven bacterial strains were undetectable after 10 min of contact with N0.001E5, N0.001E10, N0.01E5, and N0.01E10 solutions.

Non-electrolysed solutions with 0.001% and 0.01% NaCl did not inhibit the growth of bacteria. The logarithmic reductions depended on the treated bacterial strains, electrolysis, and the presence of sodium chloride in electrolysis process. For each strain, a significant difference (P ≤ 0.05) was found between the populations treated with electrolysed solutions and with non-electrolysed solutions. The bactericidal activity of the examined electrolysed solutions resulted from their physicochemical properties (Table 1), specifically high oxidation-reduction potential (863-1049 mV), low pH (2.73-3.70), and presence of free chlorine (0.37-8.45 mg/L).

The mechanism of the antimicrobial activity of the tested electrolysed solutions, morphological changes of E. coli and S. aureus, whose growth was completely inhibited, were all analysed by scanning electron microscopy. Electrolysed acidic water was found to destroy the cellular structures of the E. coli and S. aureus (Figs 1 and 2). A bactericidal effect was evidenced, since lysis of the cells subjected to AEW was observed. Some of the cells were completely destroyed (Figs 1h, i, f; Fig. 2b). The bacterial membranes changed their shapes and formed breaks after contact with N0.001E5, N0.001E10, N0.01E5 and N0.01E10 solutions (Fig. 1c, d, f; Fig. 2b) but not with non-electrolysed solutions (Fig. 1a, b, e; Fig. 2a). The changes in the appearance of E. coli, as compared to the control, were observed after N0.001E5 solution treatment (Fig. 1c).

In the AEW treatment of pork the highest reduction (compared to the control – 4.32-4.84 CFU/cm²) of total number of microorganisms (2.1 log) was observed after spraying the meat with N0.01E10 solution (Fig. 3a). Furthermore, significantly fewer psychrotrophs (more than 1 log reduction) were recovered from meat tissue (control – 2.82-3.36 CFU/cm²) sprayed with N0.001E5, N0.001E10, and N0.01E10 solutions than from tissue sprayed with 0.01% and 0.001% of sodium chloride (Fig. 3c). The treatment with N0.01E5 and N0.01E10 solutions resulted in the reduction of the number of yeast and moulds about 2.6 log and 2.5 log, respectively (compared to the control – 3.02-3.45 CFU/cm²) (Fig. 3b).

Discussion

The electrolysis caused an increase in the ACC concentration and ORP, and a decrease in pH in the solutions. Park et al. (29) reported that the concentration of chlorine in AEW is caused by the amperage of the water ioniser, but other authors (2) showed that the amount of ACC produced during electrolysis is positively correlated with the amount of sodium chloride added, which our results confirm as well. Venkatanarayanan et al. (32) explained that the high ORP of electrolysed water is caused by oxygen release due to the rupture of the weak and unstable bond between hydroxyl and chloric radicals. Low pH in AEW probably sensitises the outer membranes of bacterial cells, allowing hypochlorous acid to enter the cells of bacteria more effectively. Our results showed that low active chlorine concentration reduced many bacterial strains. This is in agreement with the results of Kim et al. (22), who used treatment with AEW containing 10 mg/L of free chlorine. Kim’s study showed that AEW is capable of reducing the populations of E. coli O157:H7, L. monocytogenes, and B. cereus vegetative cells to undetectable levels. In addition, Zhao et al. (35) reported that E. coli strains were sensitive to chlorine and that reduction of > 7 log₁₀CFU·mL⁻¹ could be achieved with a low concentration of active chlorine. Horiba et al. (12) reported that the oxidation-reduction potential, combined with low pH and free chlorine, probably significantly reduced the population of microorganisms, which confirmed our results. According to Hirano et al. (11), electrolysed water with a neutral pH did not show bactericidal activity against B. subtilis in contrast to electrolysed water with low pH, which exerted a stronger antibacterial activity. Generally, bacteria grow in the pH range of 4-9 (9). Redox potential optimal for aerobic and anaerobic microorganisms growth, ranged between 1200 to 1800 mV, and 2200 to 2400 mV, respectively. The study of Hati et al. (9) explained that high ORP causes changes in the electron flow in bacterial cells, which results in the modification of metabolic fluxes and ATP production. Low pH may sensitis the outer membrane of microorganism cells to the entry of hypochlorous acid of bacterial cells. HOCI inhibits glucose oxidation by chlorine-oxidising sulhydryl groups of enzymes in carbohydrate metabolism. Kiura et al. (24) demonstrated that different concentrations of ACC are the main cause of the antibacterial effects of AEW. Park et al. (30) reported that after exposure to AEW containing approximately 10 mg/L of residual chlorine, the population of S. aureus decreased from 8.04 log₁₀CFU·mL⁻¹ to 3.9 log₁₀CFU·mL⁻¹. Kim et al.
(22) showed that treatment with AEW containing 10 mg/L of free chlorine reduced the populations of E. coli O157:H7, L. monocytogenes, and B. cereus vegetative cells to undetectable levels. The use of more concentrated salt solutions and prolonged electrolysis time to 10 min resulted in greater changes of rod shape (Fig. 1.d, g) as compared to the control (Fig. 1.a). These findings suggest that higher active chlorine concentration, redox potential, and lower pH influence the shape and the number of breaks in the bacterial membrane. Kiura et al. (24) postulated that the cell wall of Gram-negative bacteria is one of the targets of AEW. It deactivates bacterial enzyme, probably breaks the cell wall, penetrates into the cytoplasm, and damages inner bacterial proteins. Many authors suggested that hypochlorous acid (formed during electrolysis) can penetrate cell membranes of bacteria and enhance antimicrobial action through the oxidation of key metabolic systems (2). The use of AEW for meat was the subject of many investigations. Differently than in this study, the researchers used contaminated tissue of meat. Park et al. (27) indicated that meat inoculated with C. jejuni soaked in electrolysed water was subjected to reduction by 3 log CFU·g⁻¹. Kim et al. (23) recommended to spray-wash chicken with electrolysed water before defeathering and evisceration to reduce the potential cross-contamination. Fabrizio and Cutter’s (6) demonstrated that a 15-s spraying with electrolysed water reduced populations of L. monocytogenes, S. Typhimurium, and Campylobacter coli on pork surfaces. The results from Hinton et al. (10) showed that spraying carcasses with electrolysed water significantly lowered psychrotrophic bacteria and the number of yeast cells as compared to those sprayed with tap and chlorinated water.

In conclusion, this study revealed that AEW is an effective method to significantly reduce the presence of microbes, and indicates its potential application for meat decontamination. P. fluorescens, Y. enterocolitica, E. coli, S. aureus, B. subtilis, M. luteus, and L. monocytogenes were undetectable after contact with low-concentrated AEW. The water caused shape changes of E. coli and S. aureus. The greatest changes of bacterial shape were observed after treatment with 0.01% sodium chloride subjected to a 10-min electrolysis. Acidic electrolysed water reduces growth of P. fluorescens, which is important in chill storage of meat. In comparison to the other disinfecting agents, AEW is safe for people, animals, and the environment, and lowers the cost of disinfection.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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