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In vitro antibiofilm activity of electrochemically activated water against *Salmonella* Heidelberg biofilms on polystyrene surfaces

Atividade antibiofilme *in vitro* da água eletroquimicamente ativada contra biofilmes de *Salmonella* Heidelberg em superfícies de poliestireno

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Abstract: To guarantee food safety, poultry slaughterhouses follow rigid standards to control pathogenic bacteria and prevent spoilage. However, *Salmonella* Heidelberg remains a major public health concern because it produces biofilms that increase its survival on abiotic surfaces for long periods of time. There is a global need to identify naturally-occurring compounds to remove and prevent biofilms produced on food-contact surfaces. Electrochemically activated water (ECAW) is a potential alternative to chemical disinfectants against foodborne pathogens. The antibiofilm activity has been demonstrated on stainless steel and polyethylene, but not on polystyrene surfaces. The aim of this study was to evaluate the antibiofilm activity of ECAW against *S*. Heidelberg biofilms on polystyrene surfaces and to compare with a broad-spectrum disinfectant, an alkaline detergent, and an acid detergent. All products were tested at three concentrations for antibiofilm activity against *S.* Heidelberg at 25 and 37 °C. ECAW was effective in removing *S*. Heidelberg biofilms formed on polystyrene surfaces (56% removal). The influence of contact time, product concentration, and temperature was observed on biofilm removal by ECAW. ECAW prevented up to 54% of *S*. Heidelberg biofilms on polystyrene. ECAW presented similar, or even superior, antibiofilm activity to that of disinfectant for the prevention and removal of *S*. Heidelberg biofilms. Our findings demonstrate that ECAW is effective in removing and preventing *S*. Heidelberg biofilms on polystyrene surfaces and confirmed its potential alternative to control *S*. Heidelberg in the food production chain.

Keywords: biofilm prevention; biofilm removal; natural compound

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Resumo: Para garantir a segurança do alimento, abatedouros-frigoríficos de aves seguem protocolos rígidos para evitar a contaminação por bactérias deteriorantes e patogênicas. Entretanto, *Salmonella* Heidelberg permanece como um problema de saúde pública, uma vez que é capaz de produzir biofilme e sobreviver em superfícies abióticas por longos períodos de tempo. Existe uma necessidade mundial para a identificação de compostos naturais que sejam capazes de remover e de prevenir a formação de biofilmes em superfícies de contato com alimentos. A água eletroquimicamente ativada (ECAW) é uma alternativa potencial aos desinfetantes químicos utilizados contra patógenos de alimentos. A atividade antibiofilme da ECAW já foi demonstrada em aço inoxidável e no polietileno, mas não em superfícies de poliestireno. O objetivo deste estudo foi avaliar a atividade antibiofilme de ECAW contra os biofilmes de *S.* Heidelberg em superfícies de poliestireno e comparar com um desinfetante de amplo espectro, um detergente alcalino e um detergente ácido. Todos os produtos foram testados em três concentrações para determinar a atividade antibiofilme de ECAW contra os biofilmes de *S.* Heidelberg em superfícies de poliestireno a 25°C e a 37°C. Todos os experimentos foram realizados em triplicatas. A ECAW foi efetiva em 56% na capacidade de remoção dos biofilmes de *S.* Heidelberg formados em superfícies de poliestireno, sendo observada influência do tempo de contato, concentração do produto e temperatura. Em relação à prevenção da formação dos biofilmes, ECAW foi efetiva em 54% e apresentou resultados similares ou superiores ao desinfetante e aos detergentes avaliados. Os resultados encontrados *in vitro* demonstram que ECAW é efetiva na remoção e na prevenção de biofilmes de *S.* Heidelberg em superfícies de poliestireno. Ademais, confirmam o seu potencial para ser utilizada como uma alternativa na cadeia de produção de alimentos.

Palavras-chave: composto natural; prevenção de biofilmes; remoção de biofilmes

1. Introduction

Salmonella spp. is one of the leading causes of gastroenteritis worldwide[1]. It was the second most reported zoonosis in humans in the EU in 2021[2]. In the US, 1.35 million people get sick, 26,500 are hospitalized, and 420 die from *Salmonella* spp. infection annually[3]. An aggravating bacterial feature of several *Salmonella* serotypes is the capacity to produce biofilms on different surfaces[4]. This supports bacterial survival on abiotic surfaces and in hostile environments for long periods of time, such as slaughterhouses and food processing industries, and may be a source of food contamination[5]. This represents a risk to consumer health and results in economic losses to the industry. *Salmonella* Heidelberg is an important pathogen associated with multidrug resistant outbreaks linked to poultry foods in southern Brazil. The emergence of this serotype and its high persistence in the environment has led to increased concern among food-processing plants[6,7].

To ensure food safety, food-processing plants are routinely subjected to cleaning and disinfection processes to promote microbiological control and prevent bacterial adhesion. However, due to the increased microbial resistance, there is a global concern to identify natural compounds and evaluate their efficacy against pathogens[8,9]. Electrochemically activated water (ECAW) is a natural, cost-efective, and eco-friendly compound produced through electrolysis membranes from water, salt, and electricity. The main component of ECAW is the

hypochlorous acid (HOCl)[10], an inexpensive, available, nontoxic, noncorrosive, and practical disinfectant that eliminates pathogens and that is inherently harmless[11,12].

The antimicrobial activity of ECAW against *Salmonella* spp., *Escherichia coli*, and *Listeria monocytogenes* has been previously described[13-16]. Also, the antibiofilm activity of ECAW in preventing and removing biofilms has been demonstrated for stainless steel and polyethylene surfaces[17]. Reports on polystyrene in the literature are scarce. Polystyrene is a polymer widely used in food industry for packaging meat, dairy, and bakery products[18].

In this context, the aim of this study was to evaluate the antibiofilm activity of ECAW against preformed biofilms of *S.* Heidelberg and its capacity to prevent biofilm formation on polystyrene surfaces and to compare with a broad-spectrum disinfectant, an alkaline detergent, and an acid detergent.

2. Materials and methods

2.1 Production of electrochemically activated water

ECAW was produced in a generator (Centrego, Frome, UK) with a production capacity of 200 L/h using supply water and 0.1% sodium chloride (NaCl) solution. The free chlorine and oxidation-reduction potential (ORP) of the solution were measured using a Micro 7 Plus meter (Akso, São Leopoldo, Brazil) immediately after production. The ORP values varied from 800 mV to 900 mV, and the average concentration of free chlorine obtained in the initial ECAW solution ranged from 350 ppm to 400 ppm.

2.2 Preparation of electrochemically activated water and commercial products

Four treatments were evaluated for antibiofilm activity: (A) ECAW, (B) quaternary ammonium compound (QAC) disinfectant, (C) alkaline detergent, and (D) acid detergent. ECAW was tested at three concentrations (initial solution [350–400 ppm], 200, and 250 ppm) of free chlorine. These concentrations were selected based on a previous evaluation of the *in vitro* antimicrobial activity of ECAW[15]. The disinfectant was tested at weak (0.1%), recommended (0.2%), and strong (0.5%) concentrations. Detergents were tested at weak (0.25%), recommended (0.5%), and strong (1%) concentrations, as recommended by the manufacturer. All products were diluted in sterile distilled water.

2.3 *Salmonella* Heidelberg strains

Eight *S.* Heidelberg strains isolated from poultry sources between 2018 and 2019 were randomly selected from our stock collection for this study. These strains were previously identified and serotyped by the Oswaldo Cruz Institute Foundation (Fiocruz, Brazil). All strains were previously tested using crystal violet assay to determine their ability to produce biofilms at 25 °C (room temperature) and 37 °C (the optimum temperature for *Salmonella* growth) (data not shown). Bacterial isolates were stored at –20 °C in brain heart infusion broth (BHI;

Oxoid, Basingstoke, UK) supplemented with 15% glycerin (Synth, Diadema, Brazil). Strains were reactivated in BHI for 24 h at 37 °C and then on xylose lysine deoxycholate (XLD) agar (Oxoid) for 24 h at 37 °C.

2.4 Inoculum preparation

Colonies of *S.* Heidelberg were seeded on tryptone soy agar without glucose (TSA; Oxoid) and incubated for 24 h at 37 °C. One colony of each strain was inoculated into tryptone soy broth without glucose (TSB; Oxoid), and the tubes were incubated for 24 h at 37 °C. McFarland standard No. 1 (Probac do Brasil, Brazil) was used as a reference to adjust the turbidity of the bacterial suspension in TSB to 3×10^8 CFU/mL. A spectrophotometer SP 22 (Biospectro, Brazil) was used to measure turbidity at 620 nm, which ranged from 0.224 to 0.300. The analysis was carried out in two pools of four strains each. To prepare the pools, 200 µL of each bacterial solution was inoculated in 4.2 mL of TSB to reach a final volume of 5 mL.

2.5 Removal of formed biofilms

Aliquots of 200 µL of each pool were inoculated in triplicate into each well of a sterile 96-well flat-bottomed polystyrene microplate (Kasvi; São José dos Pinhais, Brazil), followed by incubation for 24 h at 25 and 37 °C. After incubation, the cell suspension was removed, and the microplates were washed with 250 µL of 0.85% sodium chloride solution (Synth, Diadema, Brazil) to remove planktonic cells. The formed biofilm was treated with 200 µL of each product (A, B, C, and D) at their respective concentrations for 10 and 20 min at 25 °C and 37 °C. The contents were removed from each well and washed three times with 250 µL of 0.85% sterile sodium chloride solution. The attached bacteria were fixed with 200 µL of methanol (Nuclear, Brazil) per well for 20 min. The methanol was removed and the microplates were stained with 200 µL of 2% (w/v) Hucker crystal violet (MediQuímica, Brazil) per well for 15 min. The stain was removed slowly and the plate was gently washed with tap water. The plates were then air-dried at room temperature. The biofilm was resuspended in 250 µL 33% glacial acetic acid (Nuclear, Brazil) per well. The optical density (OD) of each well was measured at 550 nm using an ELx800 Absorbance Reader (Biotek, USA).

2.6 Prevention of biofilm formation

Microplates were treated with 200 µL of each product (A, B, C, and D) at their respective concentrations, followed by incubation for 24 h at 25 and 37 °C. After incubation, the contents of the wells were removed. Wells treated with detergents (C and D) were washed with 250 µL of sterile distilled water, as recommended by the manufacturer. The microplates were then air-dried at room temperature. Then, 200 µL of each pool was added in triplicate, as previously described. The microplates were incubated for 24 h at 25 and 37 °C. After incubation, the cell suspension was removed, and the microplates were prepared as previously described. The optical density (OD) of each well was measured at 550 nm using an ELx800 Absorbance Reader (Biotek, USA).

2.7 Negative, positive, and quality controls

Controls were inoculated in triplicate and were the same for both experiments. Negative and positive controls were used for all temperatures, concentrations, and contact times. For negative control (no treatment and no biofilm) only TSB without glucose was inoculated. For positive control (no treatment) a standard strain of *S.* Enteritidis (ATCC 13076) and a strain of *S.* Heidelberg from our stock collection, previously classified as biofilm producer, were used in this study.

2.8 Measurement of biofilm prevention and removal effects

The ability of each product to remove formed biofilms or prevent biofilm formation was evaluated by determining the percentage of biomass removed or not formed in relation to the untreated control. The prevention and removal of biofilm were calculated using the following formula[19]:

$$\frac{((C - B) - (T - B))}{(C - B)} \times 100\ (\%)$$

where B is the mean absorbance per well with no treatment and no biofilm (negative control), C is the mean absorbance per well without treatment (positive control), and T is the mean absorbance per well for treated wells for each compound evaluated.

2.9 Statistical analyses

All statistical analyses were performed using GraphPad Prism with a significance level of 5%. The Student's t test was used to compare biofilm removal/prevention between the different temperatures and contact times. One-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) test, was used to detect differences in biofilm prevention and removal among concentrations and compounds.

3. Results and discussion

The antibiofilm activity of ECAW has been demonstrated against *S.* Heidelberg for stainless steel and polyethylene surfaces[17], but not for polystyrene. Considering that bacteria within biofilms are up to 1,000 times more resistant to antimicrobials than their planktonic counterparts[20], it is unlikely that antimicrobial agents are capable of completely inhibit biofilms. An antimicrobial compound can be used as a preventive control measure to stop the attachment of pathogens to abiotic surfaces, or as a curative treatment to remove the formed biofilm. Therefore, in this study, we evaluated the potential use of ECAW for biofilm removal and prevention.

For this study, we selected one QAC disinfectant and two detergents. The compounds were selected for this study based on their availability in the market and use in the poultry production chain. Disinfectants and detergents present different mechanisms of action and purposes in food processing plants. While detergents are used during the cleaning step to remove the soil from surfaces, disinfectants are used during the sanitization step to reduce bacterial loads on the surfaces[21]. Thus, the antibiofilm activity of detergents was not compared to that of disinfectant and ECAW. However, because of their importance as a previous step in the cleaning and disinfection processes, detergents were included in this study to evaluate their antibiofilm activity. The disinfectant selected for this study acts by disrupting microbial cell membranes and metabolism, and is considered a broad-spectrum chemical disinfectant[22]. Detergents facilitate the contact between water and the surface by lowering the surface tension and are used to decompose and loosen the soil from surfaces[21]. The alkaline detergent used contained sodium hydroxide (NaOH). NaOH releases hydroxyl ions, which promote the saponification of fatty acids and solubilization of proteins, making them soluble in water[23,24]. Nitric acid, main component of acid detergent, is an inorganic acid that is an oxidizing agent used to remove complex soils and scale deposits from food processing plants surfaces[21,25].

The effects of the four compounds evaluated on the *in vitro* removal of biofilms formed by *S.* Heidelberg in polystyrene surface are presented in Table 1.

Table 1. Antibiofilm activity of electrochemically activated water (ECAW) (A), disinfectant based on polyhexamethylene biguanide hydrochloride and benzalkonium chloride (B), alkaline (C) and acid (D) detergents on removal of *in vitro* formed biofilm by *Salmonella* Heidelberg at 25 and 37 °C in polystyrene surface.

Compound	Concentration	Mean (%) ± standard-deviation			
		25 °C		37 °C	
		Contact time (min)		Contact time (min)	
		10	20	10	20
ECAW	200 ppm	35.53 ± 4.49^{aAB}	51.75± 3.68^{bA}	13.63 ± 9.84^{aA}	41.24 ± 16.13^{bA}
	250 ppm	22.66 ± 0.08^{aA}	55.98 ± 4.81^{bA}	14.18 ± 4.78^{aA}	42.78± 15.38^{bA}
	Initial solution (350–400 ppm)	40.66 ± 9.96^{aB}	55.31 ± 6.38^{bA}	21.47 ± 7.79^{aA}	55.82 ± 18.48^{bA}
disinfectant	0.10%	24.57 ± 5.02^{aA}	32.70 ± 6.21^{bA}	30.23 ± 42.75^{aA}	37.33 ± 15.94^{aA}
	0.20%	25.67 ± 3.34^{aA}	37.07 ± 6.07^{bA}	33.64 ± 33.04^{aA}	42.83 ± 17.76^{aA}
	0.50%	29.44 ± 4.90^{aA}	40.88 ± 6.06^{bA}	38.18 ± 15.34^{aA}	49.29 ± 20.66^{aA}
alkaline detergent	0.25%	65.41 ± 5.47^{aA}	79.15 ± 1.75^{bA}	52.26 ± 45.22^{aA}	69.48 ± 1.03^{aA}
	0.50%	74.99 ± 3.94^{aB}	83.13 ± 3.52^{bA}	65.67 ± 8.84^{aA}	72.13 ± 0.90^{aB}
	1%	77.93 ± 0.72^{aB}	81.80 ± 2.33^{bA}	76.31 ± 39.65^{aA}	75.84 ± 1.34^{aB}

acid detergent	0.25%	11.12 ± 9.15^{aA}	20.37 ± 14.06^{aA}	15.49 ± 5.82^{aA}	26.45 ± 14.57^{aA}
	0.50%	21.16 ± 7.67^{aAB}	22.88 ± 4.25^{aA}	24.03 ± 12.19^{aAB}	25.16 ± 25.98^{aA}
	1%	26.09 ± 6.61^{aB}	30.71 ± 5.25^{aA}	35.64 ± 13.30^{aB}	40.77 ± 11.99^{aA}

Different lowercase letters on the same line indicate statistically significant differences ($p<0.05$) between contact times (10 min and 20 min) for the same compound, concentration, and temperature. Different capital letters in the same column indicate statistically significant differences ($p<0.05$) among the concentrations for the same product, contact time, and temperature.

In this study, in general, the contact time was important to reducing bacterial adhesion, except for acid detergent. This influence was observed for ECAW at all temperatures and concentrations ($p<0.05$). For alkaline detergent and disinfectant, significant differences ($p<0.05$) were observed at 25 °C for all concentrations. In all cases, biofilm removal was significantly higher ($p<0.05$) after 20 min of contact. The influence of contact time on ECAW activity was previously demonstrated in planktonic cells of S. Heidelberg[17]. It is expected that increasing contact time may result in increased antibiofilm activity, regardless of the compound or bacterial species evaluated[8,26]. However, it is noteworthy that increased contact time implies longer cleaning and disinfection processes in food-processing plants.

The antibiofilm activity of chemical disinfectants depends on their concentration. The reduction of biofilm cells increases with higher disinfectant concentrations[8]. In this study, increasing the product concentration significantly increased bacterial removal at least at one temperature for ECAW and both detergents. A significant difference ($p<0.05$) was observed for ECAW at 25 °C after 10 min of contact. In this case, 250 ppm of the product resulted in a significant ($p<0.05$) lower reduction in biofilm removal than stock solution. Similarly, alkaline detergent at 0.25% resulted in the lowest biofilm removal ($p<0.05$) after 10 and 20 min of contact at 25 °C and 37 °C, respectively. Finally, the acid detergent at 1% removed significantly ($p<0.05$) more biofilm than the lowest concentration (0.25%) after 10 min of contact at both temperatures.

The influence of temperature was observed for ECAW after 10 min of contact and for alkaline detergent after 20 min of contact. In both cases, the biofilm removal was significantly higher ($p<0.05$) at 25 °C than at 37 °C. Biofilm formation by *Salmonella* isolates is strongly influenced by incubation temperatures[27], and previous studies have demonstrated the influence of temperature on biofilm removal and prevention by several microorganisms[8,27,28]. The expression of some components required for biofilm production, such as curli and cellulose, occurs mainly at temperatures ranging from 20 to 30 °C, and at higher temperatures there is an increased bacterial growth rate that may affect biofilm production[27,29]. Furthermore, a decrease in the treatment temperature is usually followed by a decrease in the efficiency of disinfectant compounds[30]. Thus, in this study, temperature was expected to influence bacterial removal and/or prevention. However, temperature did not influence biofilm removal in almost all cases evaluated.

Comparisons were also made between disinfectant and ECAW and between alkaline and acid detergents. For comparison, equivalent concentrations (low, recommended/medium, and high) were considered for each temperature. Alkaline detergent presented higher

(p<0.05) biofilm removal ability than acid detergent, regardless of the concentration, time of contact, or temperature, except for 20 min of contact at 25 °C (medium) and 10 min of contact at 37 °C (low). ECAW showed higher (p<0.05) biofilm removal than disinfectant at 25 °C for low and high concentrations, regardless of the time of contact. The results were similar for the other conditions (p>0.05).

The effects of the four compounds on the prevention of biofilm formation by *S.* Heidelberg are presented in Table 2.

Table 2. Antibiofilm activity of electrochemically activated water (ECAW) (A), disinfectant based on polyhexamethylene biguanide hydrochloride and benzalkonium chloride (B), alkaline (C), and acid (D) detergents on the prevention of biofilm formation by *Salmonella* Heidelberg at 25 and 37 °C.

Compound	Concentration	Mean (%) ± standard-deviation	
		Temperature (°C)	
		25	37
ECAW	200 ppm	43.28 ± 13.58^{aA}	39.54 ± 1.53^{aA}
	250 ppm	41.43 ± 48.49^{aA}	42.65 ± 2.82^{aA}
	Initial solution (350–400 ppm)	48.07 ± 25.17^{aA}	53.71 ± 9.13^{aB}
disinfectant	0.10%	33.49 ± 9.94^{aA}	47.33 ± 7.13^{bA}
	0.20%	38.09 ± 10.83^{aA}	53.43 ± 1.87^{bA}
	0.50%	39.84 ± 16.06^{aA}	54.67 ± 1.97^{bA}
alkaline detergent	0.25%	20.71 ± 2.46^{aA}	19.63 ± 15.76^{aA}
	0.50%	26.39 ± 19.44^{aA}	22.72 ± 22.80^{aA}
	1%	48.54 ± 12.16^{aB}	50.69 ± 6.02^{aB}
acid detergent	0.25%	21.70 ± 18.81^{aA}	43.80 ± 8.83^{bA}
	0.50%	36.27 ± 6.13^{aAB}	43.44 ± 7.61^{aA}
	1%	39.49 ± 1.46^{aB}	45.68 ± 8.53^{aA}

Different lowercase letters on the same line indicate statistically significant differences (p<0.05) between the temperatures for the same product and concentration. Different capital letters in the same column indicate statistically significant differences (p<0.05) among the concentrations for the same product and temperature.

A significant (p<0.05) influence of temperature was observed for acid detergent at 0.25% and for disinfectant at all concentrations. In both cases, biofilm prevention was significantly higher (p<0.05) at 37 °C than at 25 °C. Regarding the effect of the compound concentration, a significant difference (p<0.05) was observed at 37 °C for ECAW, and alkaline detergent. At 25 °C, significant differences (p<0.05) were observed for both detergents. In all cases, the highest concentration presented significantly (p<0.05) higher biofilm prevention than the lower concentration.

Similar to the biofilm removal assay, comparisons were made between the compounds using equivalent concentrations (low, medium, and high) at each temperature. The alkaline detergent showed higher (p<0.05) biofilm prevention than acid detergent at low concentrations at both temperatures. At 37 °C, the alkaline detergent presented higher (p<0.05) biofilm prevention than the acid detergent at a medium concentration. ECAW presented

similar (p>0.05) biofilm prevention compared to disinfectant, regardless of concentration and temperature.

Regarding biofilm removal and prevention, both detergents reduced bacterial cell load. Biofilm removal or prevention is not the main function of detergents; however, these results demonstrate the importance of the cleaning step during cleaning and disinfection. The antibiofilm activity of alkaline and acid detergents has been previously demonstrated for *Salmonella*, *E. coli*, *Vibrio parahaemolyticus*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* on several surfaces[31-35]. At higher concentrations, alkaline detergent removed/prevented more biofilm than the acid detergent. This result may be explained by the biofilm composition. Acid detergents act mainly on minerals, while alkaline detergents act on proteins and lipids, which are the main components of biofilms[36]. Furthermore, the pH of the solution plays an important role in the removal of biofilms using detergents. Previous studies demonstrated that NaOH solutions at pH 11.3 were effective in removing *Staphylococcus aureus* biofilms, but this was not observed with HCl solutions at pH 2.5[37].

In the present study, ECAW presented similar, or even superior, antibiofilm activity to that of disinfectant for the prevention and removal of *S.* Heidelberg biofilms. HOCl is the main component of ECAW; thus, its effectiveness depends on the HOCl concentration, which is related to the solution pH[38,39]. ECAW usually presents a greater bactericidal action at low pH owing to the chemical properties of the outer membrane of bacterial cells, which allow HOCl to be internalized[40,41]. However, changes in the pH of the solution may affect ECAW activity. For example, ECAW loses its antibiofilm activity at a pH ranging from 2.5 to 3.5[37], because biofilms constitute a diffusion barrier to bactericidal compounds. Thus, its antimicrobial activity is more complex against biofilms than against planktonic cells[39,42].

Regardless of the antimicrobial agent, the success of biofilm control is directly associated with adequate cleaning and disinfection procedures. Thus, it is important to prevent biofilm formation by removing attached bacteria at the early stages of biofilm formation[43]. Most products fail to control *Salmonella* biofilms after four days of maturation, because mature biofilms usually exhibit greater resistance to antimicrobial agents[33,44]. Furthermore, the presence of organic matter may influence disinfectant application[45]. In this context, the use of ECAW can be an important tool to inhibit bacterial attachment to surfaces and to prevent biofilm formation. After cleaning and disinfection processes, ECAW remains acting on the surface, preventing the adhesion of bacterial cells. However, until now, there is no authorization for ECAW use as a preventive method in Brazil.

4. Conclusion

The results demonstrated the antibiofilm activity of ECAW on polystyrene surfaces, which was enhanced with longer contact times and higher product concentrations, as well as for the other products evaluated. In some cases, ECAW showed greater biofilm removal than traditional disinfectant. Therefore, this technology presents a potential alternative for

controlling *S.* Heidelberg in the food production chain. Further analysis may include investigating the interaction between ECAW and alkaline detergents to enhance antibiofilm activity.

Declaration of conflicts of interest

The authors have no relevant financial or non-financial interests to disclose.

Author contributions

Conceptualization: D. E. Wilsmann, T. Q. Furian, K. A. Borges, A. C. Martins and V. P. Nascimento. *Data curation*: D. E. Wilsmann and T. Q. Furian. *Formal analysis*: D. E. Wilsmann and T. Q. Furian. *Funding acquisition*: D. E. Wilsmann, A. C. Martins, and V. P. Nascimento. *Project management*: D. E. Wilsmann, T. Q. Furian, A. C. Martins, D. T. Rocha, H. L. S. Moraes, and V. P. Nascimento.. *Methodology*: D. E. Wilsmann, D. Carvalho, G. Z. Chitolina, and V. Lucca. *Supervision*: A. C. Martins, D. T. Rocha, H. L. S. Moraes, and V. P. Nascimento. *Investigation*: D. E. Wilsmann, T. Q. Furian, D. Carvalho, G. Z. Chitolina, V. Lucca, and K. A. Borges. *Visualization*: D. E. Wilsmann and T. Q. Furian. *Writing (original draft)*: D. E. Wilsmann, T. Q. Furian, and K. A. Borges. *Writing (proofreading and editing)*: D. E. Wilsmann, T. Q. Furian, and K. A. Borges.

References

1. World Health Organization (WHO). Food Safety. [cited 2023 Jan 07]. Available from: https://www.who.int/news-room/fact-sheets/detail/food-safety

2. European Food Safety Authority (EFSA). *Salmonella* the most common cause of foodborne outbreaks in the European Union. [cited 2022 Nov 15]. Available from: http://www.efsa.europa.eu/en/news/salmonella-mostcommon-cause-foodborne-outbreaks-european-union

3. Centers for Diseases Control and Prevention (CDC). *Salmonella*. [cited 2022 Feb 07]. Available from: https://www.cdc.gov/salmonella/index.html. Accessed 07 February 2022.

4. Carvalho D, Chitolina GZ, Wilsmann DE, Lucca V, Dias de Emery B, Borges KA, Furian TQ, Salle CTP, Moraes HLS, do Nascimento VP. Adhesion capacity of *Salmonella* Enteritidis, *Escherichia coli*, and *Campylobacter jejuni* on polystyrene, stainless steel, and polyethylene surfaces. Food Microbiology. 2023;114:104280. http://doi.org/10.1016/j.fm.2023.104280

5. Flemming HC, Wingender J, Szewzyk U, Steinberg P, Rice SA, Kjelleberg S. Biofilms: an emergent form of bacterial life. Nature Reviews Microbiology. 2016;14:563–575. https://doi.org/10.1038/nrmicro.2016.94

6. Gieraltowski L, Higa J, Peralta V, Green A, Schwensohn C, Rosen H, Libby T, Kissler B, Marsden-Haug N, Booth H, Kimura A, Grass J, Bicknese A, Tolar B, Defibaugh-Chávez S, Williams I, Wise M. National outbreak of multidrug resistant *Salmonella* Heidelberg infections linked to a single poultry company. PLoS One 2016;11:e0162369 https://doi.org/10.1371/journal.pone.0162369

7. Voss-Rech D, Kramer B, Silva VS, Rebelatto R, Abreu PG, Coldebella A, Vaz CSL. Longitudinal study reveals persistent environmental *Salmonella* Heidelberg in Brazilian broiler farms. Veterinary Microbiology. 2019;233:118-123 http://doi.org/10.1016/j.vetmic.2019.04.004

8. Carvalho D, Menezes R, Chitolina GZ, Kunert-Filho HC, Wilsmann DE, Borges KA, Furian TQ, Salle CTP, Moraes HLS, Nascimento VP. Antibiofilm activity of the biosurfactant and organic acids against foodborne pathogens at different temperatures, times of contact, and concentrations. Brazilian Journal of Microbiology. 2022;53:1051-1064 https://doi.org/10.1007/s42770-022-00714-4

9. Crouzet J, Arguelles-Arias A, Dhondt-Cordelier S, Cordelier S, Pršić J, Hoff G, Mazeyrat-Gourbeyre F, Baillieul F, Clément C, Ongena M, Dorey S. Biosurfactants in plant protection against diseases: rhamnolipids and lipopeptides case study. Frontiers in Bioengineering and Biotechnology. 2020;8:1-11 https://doi.org/10.3389/fbioe.2020.01014

10. Wessels K, Rip D, Gouws P. *Salmonella* in chicken meat: consumption, outbreaks, characteristics, current control methods and the potential of bacteriophage use. Foods. 2021;10:1742 https://doi.org/10.3390%2Ffoods10081742

11. Block MS, Rowan BG. Hypochlorous Acid: A Review. Journal of Oral Maxillofacial Surgery. 2020 ;78(9):1461-1466. https://doi.org/10.1016%2Fj.joms.2020.06.029

12. Nature's own powerful, non-toxic disinfectant. British Dental Journal. 2018;224,553. https://doi.org/10.1038/sj.bdj.2018.302

13. Al-Holy MA, Rasco BA. The bactericidal activity of acidic electrolyzed oxidizing water against *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on raw fish, chicken, and beef surfaces. Food Control. 2015;54:317–321 https://doi.org/10.1016/j.foodcont.2015.02.017

14. Luo K, Oh D. Inactivation kinetics of *Listeria monocytogenes* and *Salmonella* enterica serovar Typhimurium on fresh-cut bell pepper treated with slightly acidic electrolyzed water combined with ultrasound and mild heat. Food Microbiology. 2016;53:165–171 https://doi.org/10.1016/j.fm.2015.09.014

15. Wilsmann DE, Carvalho D, Chitolina GZ, Borges KA, Furian TQ, Martins AC, Webber B, Nascimento VP. Electrochemically-activated water presents bactericidal effect against *Salmonella* Heidelberg isolated from poultry origin. Foodborne Pathogens and Disease. 2020;17:228-233 https://doi.org/10.1089/fpd.2019.2682

16. Zang YT, Li BM, Bing S, Cao W. Modeling disinfection of plastic poultry transport cages inoculated with *Salmonella* Enteritidis by slightly acidic electrolyzed water using response surface methodology. Poult Science. 2018;94:2059–2065 https://doi.org/10.3382/ps/pev188

17. Wilsmann DE, Furian TQ, Carvalho D, Chitolina GZ, Lucca V, Emery BD, Borges KA, Martins AC, Pontin KP, Salle CTP, de Souza Moraes HL, do Nascimento VP. Antibiofilm activity of electrochemically activated water (ECAW) in the control of *Salmonella* Heidelberg biofilms on industrial surfaces. Brazilian Journal of Microbiology. 2023;54(3):2035-2045 http://doi.org/10.1007/s42770-023-01005-2

18. Vallejos S, Trigo-López M, Arnaiz A, Miguel Á, Muñoz A, Mendía A, García JM. From Classical to Advanced Use of Polymers in Food and Beverage Applications. Polymers (Basel). 2022;14(22):4954. http://doi.org/10.3390/polym14224954

19. Pitts B, Hamilton MA, Zelver N, Stewart PS. A microtiter-plate screening method for biofilm disinfection and removal. Journal of Microbiological Methods. 2003;54:269–276. https://doi.org/10.1016/s0167-7012(03)00034-4

20. Potera C. Antibiotic resistance: biofilm dispersing agent rejuvenates older antibiotics. Environmental Health Perspectives. 2010;118:A288 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2920928/

21. Food and Agriculture Organization (FAO). Slaughterhouse cleaning and sanitation. [cited 2022 Jun 07]. Available from: https://www.fao.org/3/x6557e/X6557E00.htm#TOC

22. Chindera K, Mahato M, Sharma AK, Horsley H, Kloc-Muniak K, Kamaruzzaman NF, Kumar S, McFarlane A, Stach J, Bentin T, Good L. The antimicrobial polymer PHMB enters cells and selectively condenses bacterial chromosomes. Science Reports. 2016;6:23121 https://doi.org/10.1038/srep23121

23. Andrade NJ. Higiene na indústria de alimentos: avaliação e controle de adesão e formação de biofilmes bacterianos. São Paulo: Varela; 2008. 412 p. (Portuguese)

24. Eazychem. The benefits of using an alkaline detergent. [cited 2022 May 09]. Available from: https://www.eazychem.co.uk/benefits-using-alkaline-detergent

25. Emery BD, Chitolina GZ, Qadir MI, Furian TQ, Borges KA, Moraes HLS, Salle CTP, Nascimento VP. Antimicrobial and antibiofilm activity of silver nanoparticles against *Salmonella* Enteritidis. Brazilian Journal of Microbiology. 2023;54:285–292 https://doi.org/10.1007/s42770-022-00868-1

26. Borges A, Saavedra MJ, Simões M. The activity of ferulic and gallic acids in bioflm prevention and control of pathogenic bacteria. Biofouling. 2012;28:755–767 https://doi.org/10.1080/08927014.2012.706751

27. Borges KA, Furian TQ, Souza SN, Menezes R, Tondo EC, Salle CTP, Moraes HLS, Nascimento VP. Biofilm formation capacity of *Salmonella* serotypes at different temperature conditions. Pesquisa Veterinária Brasileira. 2018;38:71–76 https://doi.org/10.1590/1678-5150-PVB-4928

28. Kim W, Kim S, Kang D. Thermal and non-thermal treatment effects on *Staphylococcus aureus* biofilms formed at different temperatures and maturation periods. Food Research International. 2020;137:109432 https://doi.org/10.1016/j.foodres.2020.109432

29. Gemba M, Rosiak E, Nowak-Życzyńska Z, Kałęcka P, Łodykowska E, Kołożyn-Krajewska D. Factors influencing biofilm formation by *Salmonella enterica* sv. Typhimurium, *E. cloacae*, *E. hormaechei*, *Pantoea* spp., and *Bacillus* spp. isolated from human milk determined by PCA analysis. Foods. 2022;11:3862. https://doi.org/10.3390/foods11233862

30. Yuan L, Sadiq FA, Wang N, Yang Z, He G. Recent advances in understanding the control of disinfectant-resistant bioflms by hurdle technology in the food industry. Critical Reviews in Food Science and Nutrition. 2021;61:3876-3891. https://doi.org/10.1080/10408398.2020.1809345

31. Danyluk MD, Friedrich LM, Dunn LL, Zhang J, Ritenour MA. Reduction of *Escherichia coli*, as a surrogate for *Salmonella* spp., on the surface of grapefruit during various packingline processes. Journal of Food Microbiology. 2019;78:188–193. https://doi.org/10.1016/j.fm.2018.10.014

32. Elexson N, Afsah-Hejri L, Rukayadi Y, Soopna P, Lee HY, Zainazor TCT, Ainy MN, Nakaguchi Y, Mitsuaki N, Son R. Effect of detergents as antibacterial agents on biofilm of antibiotics-resistant *Vibrio parahaemolyticus* isolates. Food Control. 2014;35:378–385 https://doi.org/10.1016/j.foodcont.2013.07.020

33. Gosling RJ, Mawhinney I, Vaughan K, Davies RH, Smith RP. Efficacy of disinfectants and detergents intended for a pig farm environment where *Salmonella* is present. Veterinary Microbiology 2017;204:46–53 https://doi.org/10.1016/j.vetmic.2017.04.004

34. Hertwig AM, Prestes FS, Nascimento MS. Biofilm formation and resistance to sanitizers by *Salmonella* spp. Isolated from the peanut supply chain. Food Research International. 2022;152:110882 https://doi.org/10.1016/j.foodres.2021.110882

35. Luciano CC, Olson N, Tipple AF, Alfa M. Evaluation of the ability of different detergents and disinfectants to remove and kill organisms in traditional biofilm. American Journal of Infection Control 2016;44:e243–e249 https://doi.org/10.1016/j.ajic.2016.03.040

36. Peng D. Biofilm formation of *Salmonella*. In: Dhanasekaran D. & Thajuddin N. (Eds.), Microbial biofilms: importance and applications. IntechOpen, online. 2016. [cited 2023 Dec 04]. Available from: https://www.intechopen.com/chapters/50456

37. Sun JL, Zhang SK, Chen JY, Han BZ. Efficacy of acidic and basic electrolyzed water in eradicating *Staphylococcus aureus* biofilm. Canadian Journal of Microbiology. 2012;58:448–454. https://doi.org/10.1139/w2012-005

38. Ignatov I, Gluhchev G, Karadzhov S, Miloshev G, Ivanov N, Mosin O. Preparation of electrochemically activated water solutions (catholyte/anolyte) and studying their physical-chemical properties. Journal of Medicine, Physiology and Biophysics. 2015;13:64-78. oai:ojs.localhost:article/22411

39. Okanda T, Takahashi R, Ehara T, Ohkusu K, Furuya N, Matsumoto T. Slightly acidic electrolyzed water disrupts biofilms and effectively disinfects *Pseudomonas aeruginosa*. Journal of Infection and Chemotherapy. 2019;25:452–457 https://doi.org/10.1016/j.jiac.2019.01.014

40. Liao LB, Chen WM, Xiao XM. The generation and inactivation mechanism of oxidation-reduction potential of electrolyzed oxidizing water. Journal of Food Engineering 2007;78:1326–1332 https://doi.org/10.1016/j.jfoodeng.2006.01.004

41. Thorn RM, Lee SW, Robinson GM, Greenman J, Reynolds DM. Electrochemically activated solutions: evidence for antimicrobial efficacy and applications in healthcare environments. European Journal of Clinical Microbiology & Infectious Diseases. 2012;31:641–653 https://doi.org/10.1007/s10096-011-1369-9

42. Flemming HC, Wingender J, Szewzyk U, Steinberg P, Rice SA, Kjelleberg S. Biofilms: an emergent form of bacterial life. Nature Reviews Microbiology. 2016;14:563–575 https://doi.org/10.1038/nrmicro.2016.94

43. Craveiro S, Alves-Barroco C, Crespo MTB, Barreto AS, Semedo-Lemsaddek T. *Aeromonas* biofilm on stainless steel: efficiency of commonly used disinfectants. International Journal of Food Science & Technology. 2015;50:851–856 https://doi.org/10.1111/ijfs.12731

44. Duong H, Nguyen N, Yuk H. Changes in resistance of *Salmonella* Typhimurium biofilms formed under various conditions to industrial sanitizers. Food Control. 2013;29:236–240 https://doi.org/10.1016/j.foodcont.2012.06.006

45. Wales A, Breslin M, Davies R. Assessment of cleaning and disinfection in *Salmonella*-contaminated poultry layer houses using qualitative and semi-quantitative culture techniques. Veterinary Microbiology. 2006;116:283–293 https://doi.org/10.1016/j.vetmic.2006.04.026