Effectiveness of alkaline electrolyzed water in reducing bacterial load on surfaces intended to come into contact with food

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Abstract

Alkaline electrolyzed water (REW) is known for its cleaning action. The aim of this work was to assess REW effectiveness in reducing microbial load on surfaces intended for contact with food. Stainless-steel surfaces were experimentally contaminated, bacterial inactivation was tested before and after treatment with REW. Treatment with REW was operated spraying it on the contaminated plates until drying. Tests were conducted for *Salmonella* spp., *Listeria* spp., *Staphylococcus aureus* and *Escherichia coli*. The treatment revealed different degrees of sanitizing activity of REW on different bacterial species, with higher efficacy on *E. coli* and *Salmonella* spp. than *S. aureus*, *Listeria* spp.. Statistical analysis revealed a significant microbial load reduction ($p<0.01$) after treatment with REW, suggesting that it has a good disinfectant activity which, along with its easy and safe use, makes it a good alternative to many other more widely used disinfectants.

Introduction

Foodborne pathogens are currently estimated to be responsible for one third of human diseases in the developed world. In Europe during 2019, 27 Member States reported 5,175 food-borne outbreaks involving 49,463 cases of illness, 3,859 hospitalizations and 60 deaths: 45.7% were caused by bacteria and bacterial toxins and mainly linked to consumption of food of animal origin (EFSA and ECDC, 2021).

Although animals and humans are a major source of bacterial food contamina-tion, in the food industry, products are often biologically contaminated through contact with the surfaces of equipment, shredders, slicers, and cutting boards (Fukuzaki *et al.*, 2004; Serraino *et al.*, 2010). Procedures aiming at reducing or even eliminating pathogens from surfaces are one of the key points of an effective HACCP program in the food industry and also in controlling food contamination in homes, food markets, restaurants, health facilities and public areas (Venkitanarayanan *et al.*, 1999).

Recently, electrolyzed water (EW) has been receiving attention as a novel disinfectant and cleaning solution. Electrolyzed water is obtained from the electrolysis of a salt solution, generally NaCl (≈2 g/L). When electricity flows through the solution two types of water are generated: the cathode produces alkaline electrolyzed water (REW) containing sodium hydroxide (pH 11.6; ORP $\approx$ -795 mV), while the anode produces acidic electrolyzed water (EOW) containing hypochlorous acid (pH 2.4-2.7; ORP$\approx$1150 mV); the concentration of the residual chlorine depends on the EW machine setting (Fukuzaki *et al.*, 2004).

As a potent yet safe-to-handle sanitizer, the EOW has been easily applied into various industries, as disinfectant for food processing equipment either on various materials (stainless steel, glass) (Park *et al.*, 2002; Serraino *et al.*, 2010), or directly on food (vegetables, poultry, eggs, fish) (Athayde *et al.*, 2018; Fabrizio *et al.*, 2002; Huang *et al.*, 2008). The REW has not seen as widespread use as EOW and has been mainly considered a waste, but some research have demonstrated that it can be employed in different manners within the food industry, in particular as a cleaning solution; moreover it has been demonstrated that the combined use of REW and EOW enhances the microbial load reduction (Athayde *et al.*, 2018; Fukuzaki *et al.*, 2004; Huang *et al.*, 2008). The aim of this work was to evaluate the efficacy of the REW in reducing the microbial load on experimentally contaminated stainless-steel surfaces intended for contact with food, to define its possible use in operating conditions as a sanitizer as well as a detergent of surfaces.

Materials and methods

Alkaline electrolyzed water (REW) was obtained from Aquasol S.r.l (Bologna, Italy), generated through an electrochemical process that uses reverse osmosis water and potassium carbonate (K_2CO_3) as electrolyte. REW is made up of 99.83% pure water and 0.17% of potassium hydroxide (KOH) with a pH of 12.2-12.5 and an oxi-dation – reduction potential of -40/-90 mV. Chemical characteristics, like pH and oxidation-reduction potential (ORP), were measured with a pH-meter (FiveGo F2, Mettler – Toledo, Greifensee, Switzerland), using LE427 and LE510 electrodes to measure pH and ORP, respectively.

Four tests were performed to evaluate the disinfectant activity on *Salmonella* spp., *Listeria* spp., *Staphylococcus aureus* and *Escherichia coli*.

A mix of three different strains was used for each test (Table 1), strains were selected including one reference strain and two strains isolated from animal production chains.

For each test eight stainless steel plates were used, representing eight repetitions for each bacterial species: an area of 100 cm^2 was defined on the plates previously sterilized. Each plate was contaminated with a 10^{11} UFC/ml suspension of the above-mentioned microorganisms, using a sterile swab, to a total of about 10^4 UFC/cm^2 in the plate. The 100 cm^2 area was divided by two and, after drying, half of the plate was sampled with a sterile sponge, to evaluate the pre-treatment microbial load. REW was then applied with a low-pressure pump, left to dry for about 15 minutes and the second half of the plate was sampled through sterile sponge, to evaluate the microbial load after

treatment. In detail, sterile sponges were rubbed over the previously described areas 10 times and then put in a sterile plastic bag containing 100 mL of sterile saline solution. After mixing in a stomacher (BagMixer®, Interscience, St Nom, France) 0.1 mL of the sample obtained and 0.1 mL of four serial 10-fold dilutions were seeded in plastic Petri dishes containing PCA and incubated at 37°C for 24 h. After incubation, colonies were identified with matrix-assisted laser desorption/ionizationtime-of-flight (MALDI-TOF, Bruker, Massachusetts, USA) and then counted.

Results were expressed as log UFC/cm², and statistical analysis was made by paired T-test, one-way ANOVA and Tukey post-hoc test (p≤0.01).

Results

Treatment of artificially contaminated surfaces with REW revealed different degrees of microbial load reduction in different tests.

Overall, a 2.64 (±1.52 SD) log UFC/cm² reduction was observed, resulting in a significant (p<0.01) microbial reduction. A minimum of 0.43 log UFC/cm² in one out of the eight tests for *S. aureus* and a maximum of 5.63 log UFC/cm² in one of the eight tests for *Salmonella* spp.. In detail *Salmonella* spp. was reduced by 3.70 (±1.35 SD) log UFC/cm², *E. coli* by 3.29 (±1.35

SD) log UFC/cm², *Listeria* spp. by 2.41 (±1.22 SD) log UFC/cm², *S. aureus* by 1.16 (± 0.90 SD) log UFC/cm² on average. Details of the results are reported in Table 2 and Figure 1.

Discussion

REW is well known as detergent, dissolving fats and proteins. Some authors have suggested that using REW as pre-treatment before using disinfectants might enhance the action of the latter (Ayebah *et al.*, 2005; Fabrizio *et al.*, 2002; Jiménez-Pichardo *et al.*, 2016), therefore the cleaning action of REW could improve its disinfectant efficacy by removing organic mate-

rial that could shelter microorganisms.

In this work, treatment with REW led to a significant (p<0.01) overall reduction of microbial load compared to the pre-treatment samples. Results showed different degrees of microbial load reduction on different bacterial species. Specifically, REW showed to be significantly (p<0.01) effective on *Salmonella* spp., *E. coli, S. aureus* and *Listeria* spp.. Moreover, it appeared to be significantly (p<0.01) more effective on Gram negative bacteria (*Salmonella* spp., and *E. coli),* with an average microbial reduction of 3.49 (±1.31 SD) log UFC/cm² and a high occurrence (75%) of no microbial growth after treatment, being instead less effective on gram positive microorgan-

Table 1. Bacterial strains used and their biological matrices of origin.

| Microorganism species	Strain	Biological matrices of origin
Escherichia coli	ATCC 25922	
Escherichia coli	VeLaBac 444	Bovine faeces
Escherichia coli	VeLaBac 445	Meat
Salmonella Thyphimurium	ATCC 14020	
Salmonella Thyphimurium monophasic	118174/1	Pork sausage
Salmonella Derby	106463/1	Pork meat
Listeria monocytogenes	ATCC 15313	
Listeria innocua	257529/1	Pork sausage
Listeria innocua	257529/2	Pork meat
Staphylococcus aureus	ATCC 25923	
Staphylococcus aureus	B/122/2	Raw milk
Staphylococcus aureus	22-7-16/2	Goat skin
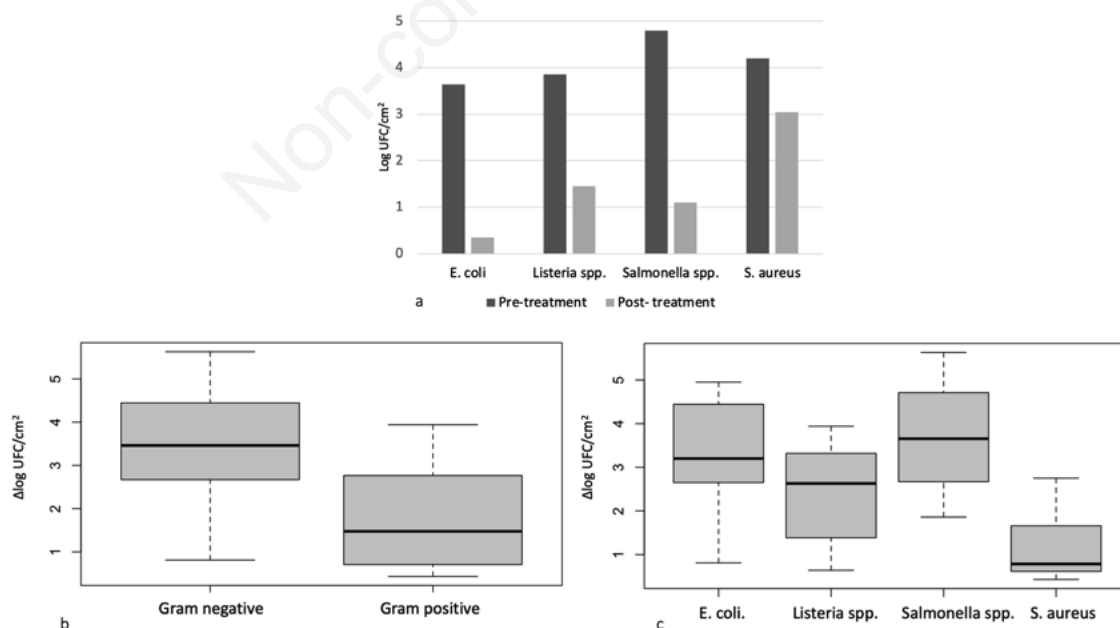
Figure 1. a) Mean Bacterial counts before and after treatment with REW for each microorganism and mean microbial load reduction values (Δlog UFC/cm²) for b) gram-negative and gram-positive bacteria, and c) each microorganism.

Table 2. Bacterial counts before and after treatment with REW, and microbial reduction expressed as mean (±SD), minimum and maximum (∆log UFC/cm²).

Microorganism	Pre – treatment Log UFC/ cm² mean (±SD)	Post- treatment Log UFC/ cm² mean (±SD)	Microbial reduction ∅Log UFC/ cm² mean (±SD)	Minimum microbial reduction ∆Log UFC/ cm²	Maximum microbial reduction ∆Log UFC/ cm²
Escherichia coli	3.64 (± 0.79)	0.35 (± 0.98)	3.29 (± 1.35)	0.81	4.95
Salmonella spp.	4.80 (± 1.29)	1.10 (± 1.58)	3.70 (± 1.35)	1.86	5.63
Listeria spp.	3.85 (± 0.49)	1.45 (± 1.61)	2.41 (± 1.22)	0.64	3.94
Staphylococcus aureus	4.20 (± 0.20)	3.04 (± 0.90)	1.16 (± 0.90)	0.43	2.75

isms (*S. aureus* and *Listeria* spp.) with an average microbial reduction of 1.78 (±1.21 SD) log UFC/cm². There's large evidence in literature of the major resistance of Gram positive bacteria to sanitizing treatments than Gram negative bacteria, and being the major difference between the two groups the thickness of the cell wall, responsible for preserving the integrity of the cell, also the results of this work suggest that the wall thickness may play a role in the REW bacterial inactivation efficacy (Koike *et al.*, 2009; Mai-Prochnow *et al.*, 2016; Vollmer *et al.*, 2008).

Other authors found similar results when testing slightly acidic electrolyzed water (SAEW) and strongly alkaline electrolyzed water, with Gram positive bacteria showing relatively more resistance to the treatment than Gram negative bacteria (Issa-Zacharia *et al.*, 2010; Koike *et al.*, 2009; Tango *et al.*, 2015). In addition, different microorganisms may have different sensibility to sanitizers, for example *Listeria* spp. is generally more resistant to chlorine than *Salmonella* spp. and *E. coli* (Burnett & Beuchat, 2000).

In literature no other work evaluated the efficacy of REW alone in reducing the microbial load on stainless steel surfaces; other authors evaluated its efficacy in association with SAEW and ultrasound to improve the sanitation of knives in the meat industry, but due to the different treatments applied we could not compare the data (Brasil *et al.*, 2020).

It is important to notice that the tests underwent microbial load conditions that are not the ones typical of working conditions. Indeed, contamination levels in the food industry are usually much lower than those considered in this work, especially considering pathogenic microorganisms. In addition, in this work it was considered just the REW action, while in operating conditions it could be associated to a mechanical bacterial removal action, that could make the treatment even more effective.

Economically REW is not competitive with the most common commercial sanitizers, yet, besides its efficacy as detergent and disinfectant, REW is safe to handle, bearing no risks for the users and has low environmental impact, being composed by 99.83% pure water and 0.17% of potassium hydroxide (KOH) and not containing environmental pollutants, moreover, its instability makes unnecessary to rinse surfaces with water after its use and hence saving time during working activities (Athayde *et al.*, 2018; Fabrizio *et al.*, 2002; Huang *et al.*, 2008). All this makes REW a suitable alternative to the most common commercial formulation for use in some particular productions process.

Conclusions

The results of this study showed its efficacy in reducing the microbial load on stainless-steel surfaces under experimental conditions, giving a good perspective on its use as sanitizing under operative conditions on surfaces intended for contact with food.

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