PAPER DETAILS

TITLE: Antibacterial Activity of Neutral Electrolyzed Water Against Listeria monocytogenes

AUTHORS: Gülay Merve Bayrakal, Ali Aydin, Çagla Sarimaden Nasri, Gürhan Çiftçioglu

PAGES: 574-579

ORIGINAL PDF URL: https://dergipark.org.tr/tr/download/article-file/3362905

Kocatepe Veterinary Journal

Kocatepe Vet J. (2023) 16(4):574-579 DOI: 10.30607/kvj.1350051

RESEARCH ARTICLE

Antibacterial Activity of Neutral Electrolyzed Water Against Listeria monocytogenes

Gülay Merve BAYRAKAL^{1*}, Ali AYDIN¹, Çağla SARIMADEN NASRİ², Gürhan ÇİFTÇİOĞLU³

¹Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, İstanbul University-Cerrahpaşa, İstanbul, Türkiye ²Eville and Jones, Lincoln, UK ³Inovatif Biotechnology Chemistry and Health Ltd., İstanbul University Technopark Bld., İstanbul, Türkiye

ABSTRACT

Inadequate cleaning and disinfection processes in the food industry are major causes of foodborne infections. Therefore, an effective cleaning process is essential for food production areas. Traditional disinfectants in the food industry have side effects like corrosion, irritation, toxicity, and residue problems. Therefore, electrolyzed water has become increasingly popular as an effective disinfectant in the food industry. Neutral electrolyzed water (NEW) contains hypochlorous acid and hypochlorite to inactivate pathogens effectively and has been proven to be stable. *Listeria monocytogenes*, a common foodborne pathogen, can be found throughout the food production chain at different points and conditions. This study examined the antimicrobial effect of NEW on *L. monocytogenes* at different concentrations, exposure times, and in vitro conditions. To assess the effectiveness of NEW, 0.1 %, 5 %, 10 %, 50 %, and 90 % concentrations were prepared, and the inactivation treatment was carried out for 30, 60, 90, and 120 min at room temperature. The results showed that at concentrations of 10 %, 50 %, and 90 %, the disinfectant significantly reduced the number of *L. monocytogenes* to undetectable levels within 30 minutes. However, about 1 log10 reduction was achieved at 0.1 % concentration in all applied times. This study reveals that NEW is a safe, environmentally friendly, and not harmful to food quality alternative to traditional disinfectants in the food industry, and recommended its increased use.

Keywords: Antimicrobial activity, Listeria monocytogenes, neutral electrolyzed water

Nötral Elektrolize Suyun Listeria monocytogenes'e Karşı Antibakteriyel Etkinliği

ÖΖ

Gıda kaynaklı enfeksiyonların başlıca nedenleri arasında gıda endüstrisindeki yetersiz temizlik ve dezenfeksiyon islemleri ver almaktadır. Bu nedenle, gıda üreten isverlerinde verinde etkin temizlik cok önemlidir. Gıda endüstrisinde kullanılan geleneksel dezenfektanların tahriş meydana getirme, korozyon, toksisite ve kalıntı oluşturma gibi yan etkileri bulunmaktadır. Bu nedenle elektrolize su, gıda endüstrisinde etkin bir dezenfektan olarak popülerlik kazanmıştır. Nötral elektrolize su (NES), patojenleri etkili bir şekilde inaktive etmek için hipokloröz asit ile hipoklorit içermekte olup, ayrıca NES'in kararlı olduğu da kanıtlanmıştır. Gıda kaynaklı yaygın bir patojen olan Listeria monocytogenes, gıda üretim zinciri boyunca farklı noktalarda ve koşullarda bulunmaktadır. Bu çalışmada, NES'in farklı konsantrasyonlarda, farklı sürelerde ve in vitro koşullarda L. monocytogenes üzerindeki antimikrobiyal etkisi incelenmiştir. NES'in etkinliğini değerlendirmek için %0.1, %5, %10, %50 ve %90'lık konsantrasyonlar hazırlanarak inaktivasyon işlemi oda sıcaklığında 30, 60, 90, 120 dakikalık sürelerde gerçekleştirilmiştir. Elde edilen sonuçlar, dezenfektanın %10, %50 ve %90 konsantrasyonlarının L. monocytogenes sayısını 30 dakika içinde tespit edilemeyen seviyelere düşürdüğünü göstermiştir. Buna karşın, %0.1 konsantrasyonda ise tüm uygulanan sürelerde yalnızca 1 log10 azalma sağlanmıştır. Bu çalışma, NES'in L. monocytogenes'i inaktive etmek için oldukça etkili olduğunu ve gıda endüstrisinde daha fazla kullanılması gerektiğini ortaya koymaktadır. Sonuç olarak, NES' in gıda endüstrisindeki geleneksel dezenfektanlara karşı güvenli, çevre dostu ve gıda kalitesine zarar vermeyen bir alternatif olduğu saptanmış olup, kullanımının artırılmasını tavsiye edilmektedir.

Anahtar Kelimeler: Antimikrobiyal aktivite, Listeria monocytogenes, nötral elektrolize su

To cite this article: Bayrakal G.M. Aydın A. Sarımaden Nasri Ç. Çiftçioğlu G. Antibacterial Activity of Neutral Electrolyzed Water Against Listeria monocytogenes. Kocatepe Vet J. (2023) 16(4): 574-579

 Submission:
 25.08.2023
 Accepted:
 05.12.2023
 Published Online:
 14.12.2023

 ORCID ID;
 GMB:
 0000-0002-2015-7182,
 AA:
 0000-0002-4931-9843,
 CSN:
 0009-0008-8202-6947
 GC:
 0000-0002-5584-517X

 *Corresponding author e-mail:
 merve.bayrakal@iuc.edu.tr
 merve.bayrakal@iuc.edu.tr
 merve.bayrakal@iuc.edu.tr

Foodborne illnesses are a major concern in the food industry. One of the main causes of foodborne infections is inadequate cleaning and disinfection in the food industry, thus proper cleaning is essential. Disinfection procedures aim to eliminate pathogens and spoilage microorganisms (Mısırlı and Aydin 2011; Pedreira et al. 2021; Yuan et al. 2021).

Disinfectants used in the food industry (chlorine compounds, organic acids, quaternary ammonium compounds, hydrogen peroxide, peroxides, amphoteric compounds, etc.) can have high costs and side effects such as corrosive defects, irritation, toxicity, and residue problems (Mısırlı and Aydin 2011; Deshmukh et al. 2019; Carrascosa et al. 2021; Yuan et al. 2021).

In recent years, electrolyzed water (EW) has been increasingly used as an effective disinfectant in the food industry (Zhao et al. 2021). EW is produced by the electrolysis of diluted salt solution (tap water and NaCl solution) in an electrolytic cell. The electrolytic cell has two sides (anode and cathode) separated by a diaphragm (Chen and Wang, 2022). Acid-electrolyzed water (AEW) produced at the anode side has low pH and high oxidation-reduction potential (Xuan and Ling 2019; Iram et al. 2021). AEW has a strong bactericidal effect but can be corrosive to processing equipment and irritating to hands (Iram et al. 2021; Jee and Ha 2021). Alkaline electrolyzed water (AIEW), or basic electrolyzed water (BEW) generated on the cathode side, has a high pH value and a low redox potential (Xuan and Ling 2019). It is commonly used as a pre-treating and cleaning agent (Xie et al. 2012).

Neutral electrolyzed water (NEW) can be produced by using several methods. One of them is (1) mixing acidic and basic electrolyzed water and others, (2) using the electrolytic system with no separation membrane, and (3) redirecting the AEW to the cathode side (Ding and Liao 2019; Xuan and Ling 2019; Iram et al. 2021).

The pH of NEW is neutral and contains two important components to inactivate pathogens: hypochlorous acid (HOCl), the most effective chlorine compound, and hypochlorite (ClO). (Cui et al. 2009; Shiroodi et al. 2021). NEW is more stable than other electrolyzed water solutions because of reduced chlorine loss (Deza et al. 2005). Compared with other traditional agents, it has some advantages: strong bactericidal and antimicrobial effects, harmless to humans and the environment, low cost, easy to use, non-corrosive, and non-irritating structure (Chen and Wang 2022).

Many studies have shown the effectiveness of NEW against various foodborne pathogens and other microbiological contaminants on food contact surfaces (Al-Qadiri et al. 2019; Possas et al. 2021; Jee and Ha 2023) and foods (Medina-Gudiño et al. 2020;

Sheng et al. 2020; Torres-Rosales at al. 2020; Hamidi et al. 2021).

Listeria monocytogenes can be found at different contact surfaces and conditions in the food production chain (Coban et al. 2019; Kara and Aslan 2021). It leads to major foodborne outbreaks and listeriosis with high mortality rates (20-30 %) (Martínez-Suárez et al. 2016; Şahin and Ayyıldız 2020). Listeriosis leads to severe sepsis, meningitis, meningoencephalitis, abortions, stillbirth, and death (Pogreba-Brown et al., 2022).

Since *L. monocytogenes* is considered one of the most important foodborne pathogens in the food industry, this current study aimed to investigate the in vitro effect of different concentrations of NEW at different periods on the viability of *L. monocytogenes*.

MATERIALS and METHODS

Bacterial strains and preparation of inoculum

NEW was obtained from commercial EW systems (Danish Clean Water, T-20 Series, Denmark). pH values and free active chlorine values of NEW were 8.5 and 499.42 mg.l-1, respectively. Concentrations of 0.1 %, 5 %, 10 %, 50 %, and 90 % of NEW were prepared using sterile deionized water immediately prior to challenge testing to demonstrate the minimum effective dose for *L. monocytogenes*. Sterile tap water was used as a control.

L. monocytogenes ATCC 19115 reference strain was used in this study's inactivation test (assay). L. monocytogenes was cultured on Tryptic Soy Agar (TSA; Oxoid, CM0131, Basingstoke, UK) plates at 37 °C for 24 h. After incubation, the bacterial cultures were transferred to a sterile tube containing 9 ml of sterile saline (0.9 % NaCI), and the suspensions were adjusted to a 0.5 McFarland (McF) of (approx. 1.5 x 10^8 CFU/ml). 1 ml suspensions were cultured on TSA plates at 37 °C for 24 h to verify the McF value.

In vitro microbial challenge (assay)

One ml of each bacterial suspension was added to the test solution containing a 9 ml mixture of NEW and deionized water. In addition, as a control group, 1 ml of each bacterial suspension was added to 9 ml of sterile tap water. All tubes were vortexed and left at room temperature (21-22 °C) for 10, 30, 60, and 90 min inactivation treatment. At the end of the treatment period, 1 ml of each sample was transferred to 9 ml of neutralizing solution (Phosphate buffered saline, Oxoid, BR0014G) and left at room temperature for 5 min to stop the activity of NEW. 0.1 % peptone water (Oxoid, LP0049) was used for serial dilutions. Each serial dilution representing the test group was plated onto TSA plates and incubated at 37 °C for 24 h. Colonies on TSA plates were counted. All the experiments were performed in duplicate (TSE EN 1276, 2019).

1 ml of each bacterial suspension was added to the test solution containing a 9 ml mixture of NEW and deionized water. At the end of the treatment, 1 ml of each mixture sample was transferred to a tube containing 9 ml of neutralizing solution, then vortexed and incubated for 5 min at room temperature to stop the activity of NEW.

Statistical analyses

In calculating the study's sample size, Power was determined by taking at least 80 % and a Type-1 error of 5 % for each variable. Shapiro-Wilk (n<50) and Skewness-Kurtosis tests were used to check whether the continuous measurements in the study were normally distributed, and Parametric tests were applied because the measurements were normally distributed. "ANOVA" was used in "periods (minutes)" and "between groups" comparisons, with "two-factor, one-factor repeated" measurements. The "Bonferroni posthoc test" was used to determine the periods and groups that account for the difference. The statistical significance level was assumed to be p<0.05 in the calculations, and the SPSS (IBM SPSS for Windows, ver.26) statistical package program was used for the analyses.

RESULTS

Our study examined the antimicrobial effect of NEW on L. monocytogenes, an important foodborne pathogen. The effectiveness of the disinfectant was measured at different dosages and exposure times.

Disinfectant concentrations of 0.1 % (4.99 mg.l-1), 5 % (24.97 mg.l-1), 10 % (49.94 mg.l-1), 50 % (249.71 mg.l-1), and 90 % (449.48 mg.l-1) were used to assess the efficacy of NEW on L. monocytogenes. The effectiveness of the disinfectant is affected by the level of free active chlorine. The level of free active chlorine in NEW used in our study is 499.42 mg.l-1. The free active chlorine level at each dilution level was calculated separately, and the disinfection efficacy was determined. The effect of NEW at different concentrations and times on the surviving population

of L. monocytogenes is shown in Table 1. Table 1 shows two-way comparison results of the concentration measurements "according to Group and Period (minutes)".

According to the results of the study, a concentration of 0.1 % was not effective; a decrease of around 1 log10 CFU/ml was achieved in all minutes. The disinfectant was observed to have a strong effect at the 90th and 120th min at 5 % concentration. No statistically significant difference between the minute measurement periods was observed in the 0.1% concentration group (p=0.102). As a result; There was no significant change in the time-dependent measurements in this group. Similar; No statistically significant difference was observed between the minute measurement periods in the 5% concentration and control groups (p=0.113 and p=0.128) There was no significant change in the time-dependent measures in these groups.

At concentrations of 10 %, 50 %, and 90 %, the number of L. monocytogenes decreased to undetectable limits – as determined by plating procedures – at any time, including 30 min of exposure to NEW. In the control samples, no significant reduction in bacterial counts was achieved after 30, 60, 90, and 120 min.

In the 30th, 60th, and 90th minute of the concentration measurement; A statistically significant difference between the groups was observed (p=0.001). The groups that make up the differences are indicated with lowercase letters. In this period, the control group was found to be different from all other groups with the highest value. Despite that, In the 120th minute period, No statistically significant difference was observed "according to the groups" (p=0.056).

In the current study, the efficacy of disinfectant containing 24.97 mg.l-1 was determined at 90 min, but when the chlorine level was 49.94 mg.l-1, it decreased to 30 min. With increasing disinfectant concentration, it was observed that NEW strongly affected L. monocytogenes even in the short period of contact time. According to our results, NEW was effective for the concentrations and periods.

Table 1. Surviving population	(log10 CFU/ml)	of L. monocytogenes
-------------------------------	----------------	---------------------

Time (min)		30	60	90	120	
		*Med±SD	Med±SD	Med±SD	Med±SD	**p.
Concentrations %	0.1 %	6.98±.01 b	6.67±.04 b	7.15±.02 b	7.22±.00	.102
	5 %	3.16±.01 c	1.95±.00 c	*ND c	ND	.113
	10 %	ND d	ND d	ND c	ND	
	50 %	ND d	ND d	ND c	ND	
	90 %	ND d	ND d	ND c	ND	
	Control	7.23±.02 a	7.38±.01 a	7.41±.01 a	7.30±.00	.128
	*р.	.001	.001	.001	.056	

*ND: Not detected of viable L. monocytogenes on TSA plates

Med, median; SD, standard deviation (The significance level of the difference between the concentration groups in the same period (a,b,c: shows different groups according to Bonferroni Post Hoc test) ** The significance level of the difference between periods in the same concentration group

DISCUSSION

Listeria spp., which can cause meningitis, miscarriage, and death, is an important microorganism for the food industry. L. monocytogenes can be found in food and the environment, especially in the food industry (Buchanan et al. 2004; Kara and Aslan 2021; Sepin and Pamuk 2021). A good cleaning and disinfection process should be done to protect from Listeria spp. in terms of food and public health (Pedreira et al. 2021). NEW is at the forefront of new-generation disinfectants due to their strong bactericidal effect as well as not harming the environment, people, and animals (Al-Qadiri et al. 2019; Chen and Wang 2022). NEW is an efficient disinfectant in the food industry (Huang et al. 2008; Cui et al. 2009).

NEW has been widely used in studies on the inactivation of *L. monocytogenes* in various foods and surfaces. Ovissipour et al. (2018) reported that AEW and NEW inactivation of *L. monocytogenes* in salmon fillets were more effective, even at different temperatures. NEW had less effect on salmon protein quality than AEW. The number of surviving *L. monocytogenes* in tomatoes washed with NEW containing 89 mg.l-1 chlorine was reduced to <1 log CFU after 5 min (Deza et al. 2003). Rivera-Garcia et al. (2019) analyzed the efficacy of NEW for *L. monocytogenes* on the eggshell surfaces and reported that a reduction of 2.18 log10 CFU/egg was achieved, and no color change was observed in the eggshells.

The number of surviving L. monocytogenes on disinfected surfaces (glass and stainless steel surfaces) using NEW with an active chlorine concentration of 63 mg.l-1was determined to be 0.18-0.19 log CFU ml^{-1} in the study by Deza et al. (2005). Using NEW (64.92 mg.l-1), a reduction in L. monocytogenes biofilms on glass and stainless steel surfaces was determined, and L. monocytogenes decreased to undetectable levels in 10 min (Arevalos-Sánchez et al. 2012). Possas et al. (2021) found a reduction from 1.55 to 5.22 log CFU/cm² was achieved on stainless steel coupons. These results were consistent with present studies reporting that NEWs could be used as an effective disinfectant to inactivate L. monocytogenes in the food industry. Although a large number of studies have been conducted with NEW, many of these studies aimed to determine the efficacy of NEW in vivo. However, our results are consistent with those studies, despite the fact that the studies were conducted in vivo makes it difficult to compare their efficacy. Although our study conducted in vitro offers an advantage in terms of the direct contact of the disinfectant with bacteria but also represents a disadvantage since any bacteria present must be destroyed. In washing studies, effectiveness is expected to be lower as the number of bacteria to be controlled spreads to the surface.

In a study with acidic (pH 2.3) and basic (pH 11.5) electrolyzed water, AEW was found to be effective in inactivating *L. monocytogenes*, while BEW was not

found to be ineffective. AEW applications detected 4 log bacteria for 15 min at 4 °C, although no bacteria were detected for 10 min at 25 °C (Fabrizio and Cutter 2003). Ovissipour et al. (2015) analyzed the effectiveness of acidic and alkaline electrolyzed water in cell suspensions for 2, 4, and 6 min at room temperature. According to this study, acidic EW is more effective than alkaline EW in inactivating pure cell suspensions of L. monocytogenes. In another study, EW with an available chlorine concentration of 5-10 mg.l-1and a pH of 6 achieved a reduction of 5-6 log CFU/ml after 30, 60, and 90 sec (Rahman et al. 2012). Although the AEW effects are high, the use of NEW in the food industry is recommended due to the disadvantages (corrosion on surfaces, irritation on mucous membranes, etc.) of acidic electrolyzed water. In the current study, NEW was effective at all disinfectant concentrations and time periods by completely inactivating L. monocytogenes, while L. monocytogenes numbers survived without reduction in tap water control groups. In the present study, a concentration of 0.1 % NEW was ineffective in reducing L. monocytogenes, while concentrations of 10 %, 50 %, and 90 % reduced it to undetectable limits. The results of this study are consistent with previous studies confirming that NEW has potent antimicrobial activity against L. monocytogenes in vitro (as a pure culture). Deza et al. (2005) found that in pure cultures of L. monocytogenes, about 7 logs were reduced after 5 min exposure to NEW. In another study, L. monocytogenes was exposed to NEW (pH 6.8 and Cl 46 mg.l-1) for 30 seconds and under in vitro conditions. After treatment, a 6.1 log10 CFU/ml reduction was determined (Rivera-Garcia et al. 2019). Hamidi et al. (2021) reported that L. monocytogenes were completely inactivated by NEW in pure culture at concentrations of 50, 100, and 200 μ g/ml and in 2 min contact time. Another in vitro assay reported that L. monocytogenes levels decreased to less than 3 log CFU/ml (Torres-Rosales et al. 2020). In vitro studies demonstrated that NEW could reduce the bacterial load and exerts a bactericidal effect on L. monocytogenes.

The effectiveness of the NEW disinfectant varies depending on contact time and concentration. In the current study, the effectiveness of a 25 μ g/kg disinfectant was determined after 90 min, but it decreased to 30 min with a chlorine level of 50 μ g/kg. These results are consistent with previous studies that reported that disinfectant efficacy increased with increasing application time and concentration (Sheng et al., 2020; Hamidi et al., 2021).

CONCLUSIONS

In conclusion, this study found that neutral electrolyzed water is highly effective in the

conditional inactivation of *L. monocytogenes* in vitro. In addition, the antimicrobial activity of NEW against *L. monocytogenes* increases as the concentration and application time increase. *L. monocytogenes*, an important food industry, and public health pathogen, can be easily inactivated by NEW disinfectant applied at appropriate concentrations and contact times. The use of NEW, which is easy to use and, does not cause harm to the environment, people, and food quality, unlike traditional disinfectants in the food industry, should be increased. The efficacy of NEW against other microorganisms should be evaluated in new studies, particularly in the in vitro setting. In this way, the effectiveness of the disinfectant can be proven by further studies.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: GÇ, AA and GMB contributed to the project idea, design of the study. GMB and ÇSN performed the experiments, data analysis and wrote the final manuscript. GÇ and GMB analyzed the experimental data and discussed the research results. GMB performed literature search, drafted and wrote the first draft of the manuscript. GÇ and AA supervised the work and reviewed/edited the manuscript critically. All the authors have read and approved the finalized manuscript.

Ethics Committee Information: "This study is not subject to the permission of HADYEK in accordance with the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees" 8 (k). The data, information and documents presented in this article were obtained within the framework of academic and ethical rules."

Explanation: We have presented as a poster at the 10th Balkan Congress of Microbiology/Microbiologia Balkanica (2017).

REFERENCES

- Al-Qadiri, H. M., Smith, S., Sielaff, A. C., Govindan, B. N., Ziyaina, M., Al-Alami, N., & Rasco, B. (2019). Bactericidal activity of neutral electrolyzed water against *Bacillus cereus* and *Clostridium perfringens* in cell suspensions and artificially inoculated onto the surface of selected fresh produce and polypropylene cutting boards. Food control, 96, 212-218. https://doi.org/10.1016/j.foodcont.2018.09.019
- Arevalos-Sánchez, M., Regalado, C., Martin, S. E., Domínguez-Domínguez, J., & García-Almendárez, B. E. (2012). Effect of neutral electrolyzed water and nisin on *Listeria monocytogenes* biofilms, and on listeriolysin
 - O activity. *Food Control*, 24(1-2), 116-122. https://doi.org/10.1016/j.foodcont.2011.09.012

- Buchanan, R., R. Lindqvist, T. Ross, M. Smith, E. Todd, R. Whiting (2004). Risk assessment of *Listeria monocytogenes* in ready-to-eat foods. Microbiological Risk Assessment Series, 4. Food and Agriculture Organization of the United Nations, Rome (Italy).
- Carrascosa, C., Raheem, D., Ramos, F., Saraiva, A., & Raposo, A. (2021). Microbial biofilms in the food industry—A comprehensive review. *International Journal of Environmental Research and Public Health*, 18(04), 2014. https://doi.org/10.3390/ijerph18042014
- Chen, B. K., & Wang, C. K. (2022). Electrolyzed water and its pharmacological activities: A mini-review. *Molecules*, 27(4), 1222. https://doi.org/10.3390/molecules27041222
- Coban, A., Pennone, V., Sudagidan, M., Molva, C., Jordan, K., & Aydin, A. (2019). Prevalence, virulence characterization, and genetic relatedness of *Listeria* monocytogenes isolated from chicken retail points and poultry slaughterhouses in Turkey. *Brazilian Journal of Microbiology*, 50, 1063-1073. https://doi.org/10.1007/s42770-019-00133-y
- Cui, X., Shang, Y., Shi, Z., Xin, H., & Cao, W. (2009). Physicochemical properties and bactericidal efficiency of neutral and acidic electrolyzed water under different storage conditions. *Journal of Food Engineering*, 91(4), 582-586. https://doi.org/10.1016/j.jfoodeng.2008.10.006
- Deshmukh, S. P., Patil, S. M., Mullani, S. B., & Delekar, S. D. (2019). Silver nanoparticles as an effective disinfectant: A review. *Materials Science and Engineering: C*, 97, 954-965. https://doi.org/10.1016/j.msec.2018.12.102
- Deza, M. A., Araujo, M., & Garrido, M. J. (2005). Inactivation of *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* on stainless steel and glass surfaces by neutral electrolysed water. *Letters in applied microbiology*, 40(5), 341-346. https://doi.org/10.1111/j.1472-765X.2005.01679.x
- Deza, M. A., Araujo, M., & Garrido, M. J. (2003). Inactivation of Escherichia coli O157: H7, Salmonella enteritidis and Listeria monocytogenes on the surface of tomatoes by neutral electrolyzed water. Letters in applied microbiology, 37(6), 482-487. https://doi.org/10.1046/j.1472-765X.2003.01433.x
- Ding, T., & Liao, X. (2019). Decontamination efficacy and principles of electrolyzed water. In *Electrolyzed Water in Food: Fundamentals and Applications* (pp. 17-38). Singapore: Springer Singapore. https://doi.org/10.1007/978-981-13-3807-6_2
- Fabrizio, K. A., & Cutter, C. N. (2003). Stability of electrolyzed oxidizing water and its efficacy against cell suspensions of Salmonella Typhimurium and Listeria monocytogenes. Journal of Food Protection, 66(8), 1379-1384. https://doi.org/10.4315/0362-028X-66.8.1379
- Hamidi, R. M., Shekarforoush, S. S., Hosseinzadeh, S., & Basiri, S. (2021). Near Neutral Electrolyzed Water and Peroxyacetic Acid and Their Effect on the Survival of Escherichia coli, Salmonella Typhimurium, and Listeria monocytogenes Inoculated on Poultry Meat. Food Protection Trends, 41(4).
- Huang, Y. R., Hung, Y. C., Hsu, S. Y., Huang, Y. W., & Hwang, D. F. (2008). Application of electrolyzed water in the food industry. *Food control*, 19(4), 329-345. https://doi.org/10.1016/j.foodcont.2007.08.012
- Iram, A., Wang, X., & Demirci, A. (2021). Electrolyzed oxidizing water and its applications as sanitation and cleaning agent. *Food Engineering Reviews*, 13, 411-427. https://doi.org/10.1007/s12393-021-09278-9

- Jee, D. Y., & Ha, J. W. (2021). Synergistic interaction of tap water-based neutral electrolyzed water combined with UVA irradiation to enhance microbial inactivation on stainless steel. *Food Research International*, 150, 110773. https://doi.org/10.1016/j.foodres.2021.110773
- Jee, D. Y., & Ha, J. W. (2023). Inactivation of Escherichia coli O157: H7, Salmonella Typhimurium, and Listeria monocytogenes on stainless steel by synergistic effects of tap water-based neutral electrolyzed water and lactic acid. Food Microbiology, 112, 104233. https://doi.org/10.1016/j.fm.2023.104233
- Kara, R., & Aslan S. (2021). Investigation of Listeria monocytogenes in workers, equipment and environmets at kaymak processing plants. Food Science and Technology, 41, 449-452. https://doi.org/10.1590/fst.02620
- Martínez-Suárez, J. V., Ortiz, S., & López-Alonso, V. (2016). Potential impact of the resistance to quaternary ammonium disinfectants on the persistence of *Listeria monocytogenes* in food processing environments. *Frontiers in microbiology*, 7,

638. https://doi.org/10.3389/fmicb.2016.00638

- Medina-Gudiño, J., Rivera-Garcia, A., Santos-Ferro, L., Ramirez-Orejel, J. C., Agredano-Moreno, L. T., Jimenez-Garcia, L. F., ... & Cano-Buendia, J. A. (2020). Analysis of Neutral Electrolyzed Water antibacterial activity on contaminated eggshells with Salmonella enterica or Escherichia coli. International journal of food microbiology, 320, 108538. https://doi.org/10.1016/j.ijfoodmicro.2020.108538
- Mısırlı, F., & Aydin, A. (2011). Effectiveness of different disinfectants used in the food production facility on selected foodborne pathogens. *Kafkas Universitesi Veteriner Fakültesi Dergisi*, 17(suppl), 167-171. https://doi.org/10.9775/kvfd.2010.3500
- Ovissipour, M., Al-Qadiri, H. M., Sablani, S. S., Govindan, B. N., Al-Alami, N., & Rasco, B. (2015). Efficacy of acidic and alkaline electrolyzed water for inactivating Escherichia coli O104: H4, Listeria monocytogenes, Campylobacter jejuni, Aeromonas hydrophila, and Vibrio parahaemolyticus in cell suspensions. Food Control, 53, 117-123. https://doi.org/10.1016/j.foodcont.2015.01.006
- Ovissipour, M., Shiroodi, S. G., Rasco, B., Tang, J., & Sablani, S. S. (2018). Electrolyzed water and mildthermal processing of Atlantic salmon (Salmo salar): Reduction of *Listeria monocytogenes* and changes in protein structure. *International journal of food microbiology*, 276, 10-19. https://doi.org/10.1016/j.ijfoodmicro.2018.04.005
- Pedreira, A., Taşkın, Y., & García, M. R. (2021). A critical review of disinfection processes to control SARS-CoV-2 transmission in the food industry. *Foods*, 10(2), 283. https://doi.org/10.3390/foods10020283
- Pogreba-Brown, K., Boyd, K., Schaefer, K., Austhof, E., Armstrong, A., Owusu-Dommey, A., ... & Hoffman, S. (2022). Complications Associated with Foodborne Listeriosis: A Scoping Review. Foodborne Pathogens and Disease, 19(11), 725-743. https://doi.org/10.1089/fpd.2022.0012
- Possas, A., Pérez-Rodríguez, F., Tarlak, F., & García-Gimeno, R. M. (2021). Quantifying and modelling the inactivation of *Listeria monocytogenes* by electrolyzed water on food contact surfaces. *Journal of Food Engineering*, 290, 110287. https://doi.org/10.1016/j.jfoodeng.2020.110287
- Rahman, S. M. E., Park, J. H., Wang, J., & Oh, D. H. (2012). Stability of low concentration electrolyzed water and its sanitization potential against foodborne pathogens. *Journal of Food Engineering*, 113(4), 548-553. https://doi.org/10.1016/j.jfoodeng.2012.07.011

- Rivera-Garcia, A., Santos-Ferro, L., Ramirez-Orejel, J. C., Agredano-Moreno, L. T., Jimenez-Garcia, L. F., Paez-Esquiliano, D., ... & Cano-Buendia, J. A. (2019). The effect of neutral electrolyzed water as a disinfectant of eggshells artificially contaminated with Listeria monocytogenes. Food science & nutrition, 7(7), 2252-2260. https://doi.org/10.1002/fsn3.1053
- Sepin, Ö., & Pamuk, Ş. (2021). Afyonkarahisar'da satışa sunulan tavuk iç organlarından Salmonella spp. ve Listeria monocytogenes' in immonomagnetik seperasyon yöntemi ile izolasyonu ve izolatların antibiyotik dirençliliğinin belirlenmesi. Kocatepe Veterinary Journal, 14(2), 201-209. https://doi.org/10.30607/kvj.839629
- Sheng, L., Shen, X., Ulloa, O., Suslow, T. V., Hanrahan, I., & Zhu, M. J. (2020). Evaluation of JC9450 and neutral electrolyzed water in controlling *Listeria monocytogenes* on fresh apples and preventing cross-contamination. *Frontiers* in Microbiology, 10, 3128. https://doi.org/10.3389/fmicb.2019.03128
- Shiroodi, S., Schwarz, M. H., Nitin, N., & Ovissipour, R. (2021). Efficacy of nanobubbles alone or in combination with neutral electrolyzed water in removing *Escherichia coli* O157: H7, *Vibrio parahaemolyticus*, and *Listeria innocua* biofilms. *Food and Bioprocess Technology*, 14, 287-297. https://doi.org/10.1007/s11947-020-02572-0
- Şahin, S., & Ayyıldız, A. (2020). Sivas ilinde satılan çiğ inek ve manda sütlerinde *Listeria* spp. varlığının araştırılması. *Kocatepe Veterinary Journal*, 13(1), 19-24. https://doi.org/ 10.30607/kvj.645718
- Torres-Rosales, E., Rivera-Garcia, A., Rosario-Perez, P. J., Ramirez-Orejel, J. C., Paez-Esquiliano, D., Martinez-Vidal, S., ... & Cano-Buendia, J. A. (2020). Application of Neutral Electrolyzed Water on pork chops and its impact on meat quality. *Scientific Reports*, 10(1), 19910. https://doi.org/10.1038/s41598-020-76931-4
- **TSE EN 1276 of Turkish Standards Institution of 2019.** On Chemical disinfectants and antiseptics-Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas - Test method and requirements (phase 2, step 1)
- Xie, J., Sun, X., Pan, Y., & Zhao, Y. (2012). Combining basic electrolyzed water pretreatment and mild heat greatly enhanced the efficacy of acidic electrolyzed water against *Vibrio parahaemolyticus* on shrimp. *Food Control*, 23(2), 320-324. https://doi.org/10.1016/j.foodcont.2011.07.019
- Xuan, X., & Ling, J. (2019). Generation of electrolyzed water. Electrolyzed water in food: Fundamentals and applications. Singapore: Springer Singapore.
- Yuan, L., Sadiq, F. A., Wang, N., Yang, Z., & He, G. (2021). Recent advances in understanding the control of disinfectant-resistant biofilms by hurdle technology in the food industry. *Critical Reviews in Food Science and Nutrition*, 61(22), 3876-3891.https://doi.org/10.1080/10408398.2020.1809345
- Zhao, L., Li, S., & Yang, H. (2021). Recent advances on research of electrolyzed water and its applications. *Current Opinion in Food Science*, 41, 180-188. https://doi.org/10.1016/j.cofs.2021.03.004