Med Clin Pract. 2020;3(S1):100090

MEDICINA CLINICA PRÁCTICA

www.elsevier.es/medicinaclinicapractica

Original article

Evaluation of different type of electrolyzed water against bacterial colonization of diabetic foot ulcers: Study in vitro☆

Edy Supardi[a], Saldy Yusuf[b,*], Muh Nasrum Massi[c], Hasniati Haeruddin[d]

[a] Nani Hasanuddin College of Health Sciences Makassar, Indonesia
[b] Faculty of Nursing, Hasanuddin University, Indonesia
[c] Medicine Departement, Faculty of Medicine, Hasanuddin University, Indonesia
[d] Gema Insan akademik College of Health Sciences Makassar, Indonesia

ARTICLE INFO

Article history:
Received 29 May 2019
Accepted 15 July 2019

Keywords:
Electrolyzed water
pH water
Diabetic foot ulcer

ABSTRACT

Objective: This study aims to evaluate the effect of electrolyzed water effect on bacterial colonization on diabetic foot ulcer (DFU) patients.
Method: This is an in vitro study. Eight bacteria are incubated into 5 types of electrolyzed water (ESAW), electrolyzed weak acid water (EWAW), electrolyzed neutral water (ENAW), electrolyzed weak alkaline water (EWAlW), and electrolyzed strong alkaline water (ESAlW). Evaluations were performed 2, 24, 48 and 72 h after incubation. Data were analyzed using repeated ANOVA test used to compare the difference of electrolyzed water effect on bacterial colonization of DFU patients.
Results: ESAW (pH 2.5) significantly ($p = 0.001$) had a better bactericidal effect than EWAW, ENAW, EWAlW and ESAlW.
Conclusion: Current study confirmed the positive effect ESAW on bacterial colonization in DFU patients.

Introduction

Diabetic foot ulcers (DFUs) associated with various devastating complications, such as; long term care, high cost,[1,2] infection,[3] amputation[4,5] and mortality.[6] Therefore prompt and aggressive treatment are needed to accelerate wound healing progress, which will be prevent worsening of ulcers, maintain extremity intact and quality of life.[4]

One of essential modality in management of DFUs is wound cleansing. Wound Cleansing is one of wound care procedure.[7,8] At least normal saline and tap water are reported as main wound cleansing agent wound cleansing,[9] meanwhile the advantages of acid cleansing has less evaluated.

Previous study revealed that Hypochlorus acid improved wound healing since it has antimicrobial effect including in DFUs.[10] The benefits of pH neutral in a study of Neutralized electrolyzed water (NEW) effects that were preceded by an in vitro study showed that NEW pH 7.7 significantly reduced the number of *Aspergillus flavus* spores and *Penicillium expansum* spores.[11] Although electrolyzed alkaline water has many health benefits including improving abnormal intestinal fermentation, chronic diarrhea, gastric hyperacidity, dyspepsia, improving constipation, suppressing body fat accumulation, expelling early melamine, reducing skin damage due to ultraviolet light, modulation of immune responses and repair diabetes.[12] Despite many studies has reported the benefit of pH water, the effectiveness as wound cleansing remain unknown. Thus, the aim of current study to evaluate the effect of electrolyzed water effect on bacterial colonization on diabetic foot ulcer (DFU) patients.

Methods

Setting

This was an experiment in vitro study. The swabbing were taken from DFUs patients both inpatient-outpatient clinic (Hospital) and three home care setting (private wound care practices) by an accidental sampling technique. HbA1C > 6% and Texas university classification system (grade B), as inclusion criteria.

☆ Peer-review under responsibility of the scientific committee of the International Conference on Women and Societal Perspective on Quality of Life (WOSQUAL-2019). Full-text and the content of it is under responsibility of authors of the article.

* Corresponding author.
E-mail address: saldy_yusuf@yahoo.com (S. Yusuf).

https://doi.org/10.1016/j.mcpsp.2020.100090

Swabbing were taken using Levine technique. Culture of wound specimens through inoculation on the medium, incubated for 24 h to identify bacteria and its gram properties.

Preparation

The electrolysis process of water uses the electrolysis machine (Leveluk SD 501, Japan), to prepare five different types of electrolyzed water (EW). Status of EW were measured by pH meter (Hanna pHep Tester HI98107, Romania), and validated at the Makassar Environmental Health and Disease Control Laboratory.

In vitro

The identified bacteria were made suspended in 0.9% NaCl solution using Mcfarlan 0.5 standard, then insert 5 types of electrolyzed water (EW) into the 9 ml screw tube, sterilize. Suspension of 8 types of bacteria 1 ml were added into a tube which each contains 5 types of EW, then mixed until homogeneous. Incubation running for 2 h then put into sterile petri dish and adding medium plate count agar (PCA) to the petri dish, mix until homogeneous. After PCA solidified, incubate it into an incubator at 37 °C for 2 h. After 2 h incubation, the number of bacterial colonies that grow on the medium were calculated. The procedure were repeated until the second day (48 h) and third (72 h).

Analysis

Demography data presented in frequency table (*n*, %), while evaluation of electrolyzied watered against bacterial colonization were analyzed using repeated one way anova (SPSS, ver 22, Chicago Inc). Ethical clearance was approved from Ethical Committee, Faculty of Medicine, Hasanuddin University. No. 437/H4.8.4.5.31/PP36-KOMETIK/2017.

Table 1
Frequency distribution of participants based on demographic data.

Variabel	Total	
	n: 20	%
Age (mean, +SD)	53.1	6.7
Late adults (36–45 years old)	3	15.0
Early elderly (46–55 years old)	10	50.0
Late elderly (56–65 years)	7	35.0
Gender		
Male	14	70.0
Female	6	30.0
Religion		
Islam	19	95.0
Christian Protestant	1	5.0
Ethnic		
Bugis	12	60.0
Makassar	6	30.0
Toraja	1	5.0
Java	1	5.0
Last education		
Primary School	3	15.0
Junior High School	1	5.0
Senior High School	8	40.0
Diploma	2	10.0
Bachelor/Masters/Doctorate	6	30.0
Occupation		
Government officer/Army/Police/Lecture	8	40.0
Honorary	1	5.0
Entrepreneur	7	35.0
Farmer	1	5.0
Housewife	3	15.0

Results

Characteristics of participants

Half of participants on early elderly on age group (46–55 years) (*n*: 10, 50.0%), the mean and standard deviation of the participants' age (53.1, ±6.7), dominantly male gender (*n*: 14, 70.0%), Islam (*n*: 19, 95.0%), Bugis ethnic (*n*: 12, 60.0%), the last education for Senior High School (*n*: 8, 40.0%), government officer and self-employed (*n*: 8, 40.0%) (Table 1).

Table 2
Frequency distribution based on the participants' diabetes mellitus status.

Variabel	Total	
	n: 20	%
Duration of diabetes		
<5 years	8	40.0
5–10 years	5	25.0
>10 years	7	35.0
Glycemic Control		
There is no	3	15.0
Oral	8	40.0
Insulin	5	25.0
Oral and insulin	4	20.0
Smoking history		
Never	8	40.0
Ever	8	40.0
Active	4	20.0
HbA1C (mean, ±SD)	10.3	2.6
Blood pressure		
Sistole (mmHg) (mean, ±SD)	130.1	19.5
Diastole (mmHg) (mean, ±SD)	84.5	12.8
Height (cm) (mean, +SD)	165.9	7.4
Weight (Kg) (mean, +SD)	62.9	10.1
Body Mass Index (BMI) (kg/m^2) (mean, +SD)	22.7	2.4
BMI category		
Underweight (<18.49)	1	5.0
Normal (18.50–24.99)	16	80.0
Overweight (25.00–29.99)	3	15.0

Table 3
Frequency distribution of participants based on a history of diabetic foot ulcers and diabetic foot status.

Variables	Total	
	n: 20	%
Duration of ulcer DFU		
<1 month	7	35.0
1–2 months	11	55.0
>2 months	2	10.0
Causes of ulcers		
Unknown	8	40.0
Trauma	10	50.0
Non trauma	2	10.0
History of ulcer		
None	5	25.0
Yes	15	75.0
History of amputation		
None	15	75.0
Yes	5	25.0
Neuropathy status		
Non neuropathy	9	45.0
Neuropathy	11	55.0
Angiopathy status		
Non Angiopathy	12	60.0
Angiopathy	8	40.0

Table 4
Frequency distribution of bacterial types in participants' diabetic foot ulcer based on the wound classification of University of Texas Classification system.

The type of bacteria	University of Texas Classification system							
	Stage B Grade 1		Stage B Grade 2		Stage B Grade 3		Total	
	n = 11	%	*n* = 1	%	*n* = 8	%	*n* = 20	%
Alkaligenes faecalis	1	9.1	0	0	0	0	1	5.0
Eschericia coli	0	0	0	0	4	50.0	4	20.0
Enterobacter agglumerans	1	9.1	0	0	0	0	1	5.0
Klebsiella Sp	1	9.1	1	100	0	0	2	10.0
Proteus mirabilis	5	45.5	0	0	1	12.5	6	30.0
Proteus vulgaris	1	9.1	0	0	2	25.0	3	15.0
Providencia alkalifaesen	1	9.1	0	0	0	0	1	5.0
Providencia stuarti	1	9.1	0	0	1	12.5	2	10.0

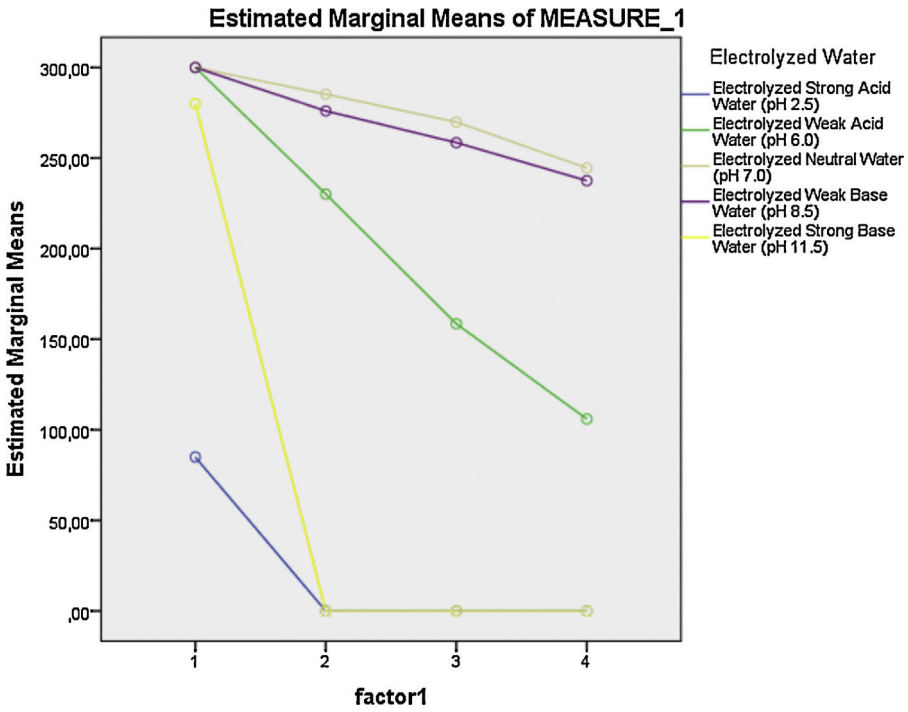

Fig. 1. Estimated effect of electrolyzed water on time bacterial colonization from the evaluation time of at 2, 24, 48, and 72 h (Factor1*: 1, 2, 3, 4).

DM status based on diabetes duration <5 years (*n*: 8, 40.0%), mean and standard deviation of HbA1C (10.3, ±2.6%), Glycemic Control by oral (*n*: 8, 40.0%), history of never smoking (*n*: 8, 40.0%), the mean and standard deviation of the participants' height (165.9 cm, ±7.4), weight (62.9 kg, ±10.1), Body Mass Index (BMI) (22.7 kg/m^2, ±2.4), the normal BMI category (18.50–24.99) (*n*: 16, 80.0%), systolic blood pressure (130.1 mmHg, ±19.5), and diastole blood pressure (84.5 mmHg, ±12.8) (Table 2).

DFU history based on wound duration 1–2 months (*n*: 11, 55.0%), causes of DFU injuries due to trauma (*n*: 10, 50.0%), there was a history of previous injuries (*n*: 15, 75.0%), no amputation history (*n*: 15, 75.0%), Neuropathy (*n*: 11, 55.0%), and angiopathy (*n*: 12, 60.0%) (Table 3).

The dominant aerobic bacterial colonies found in all participants were Proteus mirabilis (*n*: 6, 30.0%). Based on The classification of University of Texas Classification system, the dominant type of proteus mirabilis bacteria was found in stage B grade 1 (*n*: 5, 45.5%), while *Eschericia coli* bacteria were dominantly found in stage B grade 3 (*n*: 4, 50%) (Table 4).

The effect of electrolyzed water in reducing the number of bacterial colonies based on the evaluation time of 2 h, 24 h, 48 h to 72 h. This indicates bactericidal effects have occurred at 2 h after incubation (Fig. 1).

There are significant differences in the effect of electrolyzed water on bacterial colonization, where ESAW (pH 2.5) significantly (p = 0.001) which confirmed had a better bactericidal effect compared to EWAW, ENAW, EWAlW and ESAlW. However, between ENW pH 7.0 and EWAlW pH 8.5 there seemed no significant difference, where the p value = 0.951 (Table 5).

Discussion

Electrolyze water produces strong and weak acid and base solutions.[13] PH levels Electrolyzed neutral water is located at 7 to 8. Electrolyzed strong acid water has a pH value of 3 to 2, while electrolyzed strong alkaline water has a pH value of 10 to 13. pH values between 5.0 to 6.5 and 8.0 to 10 each is slightly acidic and slightly alkaline Electrolyzed water has strong bactericidal, fungicidal and virucidal effects in various fields such as medicine.[14] Determination of in vitro microbiology whether antibacterial agents are bactericidal or bacteriostatic is influenced by growth conditions, bacterial density, duration of test, and degree of decrease in bacterial count. antibacterial is better described as potentially bactericidal and bacteriostatic.[15] In this study ESAW (pH 2.5) had a better bactericidal effect from the first 2 h to 72 h after the incubation of proteus mirabilis, *Eschericia coli*, proteus vulgaris, alkaligenes faecalis and

Table 5
Analysis of the effect of electrolyzed water on bacterial colonization.

Electrolyzed water	Mean difference	95% CI	*p* value
Electrolyzed strong acid water (ESAW) (pH 2.5)			
ESAW (pH 2.5) v.s EWAW (pH 6.0)	−177.4	(−)204.9–(−)149.8	0.001
ESAW (pH 2.5) v.s ENW (pH 7.0)	−253.7	(−)281.2–(−)226.1	0.001
ESAW (pH 2.5) v.s EWAlW (pH 8.5)	−246.8	(−)274.3–(−)219.2	0.001
ESAW (pH 2.5) v.s ESAlW (pH 11.5)	−48.8	(−)76.3–(−)21.2	0.001
Electrolyzed weak acid water (EWAW) (pH 6.0)			
EWAW (pH 6.0) v.s ENW (pH 7.0)	−76.3	(−)103.8–(−)48.7	0.001
EWAW (pH 6.0) v.s EWAlW (pH 8.5)	−69.3	(−)96.9–(−)41.8	0.001
EWAW (pH 6.0) v.s ESAlW (pH 11.5)	128.7	101.1–156.2	0.001
Electrolyzed neutral water (ENW) (pH 7.0)			
ENW (pH 7.0) v.s EWAlW (pH 8.5)	6.9	(−)20.7–34.5	0.951
ENW (pH 7.0) v.s ESAlW (pH 11.5)	204.9	177.3–232.6	0.001
Electrolyzed weak alkaline water (EWAlW) (pH 8.5)			
EWAlW (pH 8.5) v.s ESAW (pH 11.5)	198.0	170.4–225.6	0.001
Electrolyzed weak acid water (ESAlW) (pH 11.5)			
ESAlW (pH 11.5) v.s ESAW (pH 2.5)	48.8	21.2–76.3	0.001
ESAlW (pH 11.5) v.s EWAW (pH 6.0)	−128.7	(−)156.2–(−)101.1	0.001
ESAlW (pH 11.5) v.s ENW (pH 7.0)	−204.9	(−)232.5–(−)177.3	0.001
ESAlW (pH 11.5) v.s EWAlW (pH 8.5)	−198.0	(−)225.6–(−)170.4	0.001

enterobacter compared to EWAW (pH 6.0), ENW (pH 7.0), EWAlW (pH 8.5), and ESAlW (pH 11.5). The bactericidal potential possessed by ESAW (pH 2.5) in this study was due to the presence of low pH levels, ORP and residual chlorine. Although EW's bactericidal activity and its mechanism of action are still not fully understood,[14] some scientists consider the presence of chlorine in EW as the main factor responsible for bactericidal activity, while others consider ORP to be a major factor.[16] Chlorine concentration (Cl_2), ORP and pH can affect the bactericidal effectiveness of EW.[17] Low pH is known to be responsible for decreasing bacterial production and making bacterial cells more susceptible to dynamic chlorine.[19] Bacterial cell inactivation occurs because of a high ORP (1150 mV) in acid EW, causing oxidation on the cell surface, damaging various cell layers, and disrupting the metabolic pathway in the cell. In principle, low pH and high ORP in EW Acid acts synergistically with chlorine in reactivating microorganisms.[20]

The results of this study are in line with previous study which confirmed electrolyzed water acid (pH 2.6) has a disinfecting effect in reducing bacteria,[21] thus electrolyzed water acid is effective in reducing bacteria,[22] and has a higher bactericidal effect than EW pH 5.6–5.7 and pH 8.[23] In addition, the bacterial colonies in EWAW (pH 6.0) is lower than ESAW (pH 2.5) and ESAlW (pH 11.5). Nevertheless, the bactericidal effect possessed by EWAW (pH 6.0) from the first 2 h to 72 h after the bacteria was incubated is still better than ENW (pH 7.0) and EWAlW (pH 8.5). Active chlorine in the form of hypochlorous acid (HOCl), which dominates when the pH of the solution is 5.0–6.5. HOCl is dissociated with hypochlorite ions (OCl^-) at high pH or chlorine gas (Cl_2) at low ph.[18] This study are in line with previous study that slightly electrolyzed water acid (pH 6.0–6.5) was effective in deactivating *Eschericia coli* and *Staphylococcus aureus*.[24] One study also reported that slightly acidic electrolyzed water pH range 5.6–5.7 significantly has a bactericidal effect in reducing *Eschericia coli*.[23] Using a slightly acidic electrolyzed water (pH 6.3) has a bactericidal effect in deactivating bacteria[25]

Electrolyzed neutral water and electrolyzed weak alkaline water in this study have the same lower bactericidal effect on the amount of bacterial colonization compared to other EW types. This is because the decrease in the average number of colonies in these two types of EW is not significant. The optimal pH level for bacterial growth is 4–9.[18,26] The ability to deactivate all organisms decreases at pH 9.[14]

Electrolyzed strong alkaline water (pH 11.5) also has a better bactericidal effect than EWAW (pH 6.0), ENW (pH 7.0), EWAlW (pH 8.5), but is no better than the bactericidal effect of ESAW (pH 2.5) because its bactericidal activity begins at 24 h evaluation. The results of this study are consistent with previous finding, that alkaline EW (pH 11.3) has bacterial reduction ability.[27]

However, with a small sample size and study design, caution must be applied, as the findings might not be different in clinical study, particularly potential negative effect on wound tissue which need more investigation.

Conclusion

Electrolyzed strong acid water (pH 2.5) has a better bactericidal effect compared to other types of electrolyzed water. Electrolyzed weak acid water (pH 6.0) has a better bactericidal effect than EW pH 7 and EW pH 8.5 There is no difference in the effect of electrolyzed neutral water (pH 7.0) with electrolyzed weak alkaline water (pH 8.5) on the reduction of bacterial colonization and both have an effect bactericidal which is worse than other EW. Electrolyzed strong alkaline water (pH 11.5) has a better bactericidal effect than EW pH 6.0, EW pH 7.0 and EW pH 8.5.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

Current study granted from Ministry of Research and Technology of the Republic of Indonesia as the magister scholarship.

References

1. Cárdenas MK, Mirelman AJ, Galvin CJ, Lazo-Porras M, Pinto M, Miranda JJ, et al. The cost of illness attributable to diabetic foot and cost-effectiveness of secondary prevention in Peru. *BMC Health Serv Res* 2015;**15**:483, http://dx.doi.org/10.1186/s12913-015-1141-4.
2. Keskek SO, Kirim S, Yanmaz N. Estimated costs of the treatment of diabetic foot ulcers in a tertiary hospital in Turkey. *Pak J Med Sci* 2014;**30**:968–71, http://dx.doi.org/10.12669/pjms.305.5182.
3. Muduli ICPPA, Panda C, Behera NC. Diabetic foot ulcer complications and its management – a medical college-based descriptive study in Odisha, an Eastern State of India. *Indian J Surg* 2015;**77**(Suppl. 2):270–4, http://dx.doi.org/10.1007/s12262-012-0791-2.
4. Cancelliere P. Current epidemiology of diabetic foot ulcers. *Int J Diab* 2016;**1**:1–3.

5. Wang DD, Jamjoom RA, Alzahrani AH, Hu FB, Alzahrani HA. Prevalence and correlates of lower-extremity amputation in patients with diabetic foot ulcer in Jeddah Saudi Arabia. *Int J Low Extrem Wounds* 2016;**15**:26–33, http://dx.doi.org/10.1177/1534734615601542.
6. Chammas NK, Hill RL, Edmonds ME. Increased mortality in diabetic foot ulcer patients: the significance of ulcer type. *J Diab Res* 2016;**2016**:2879809, http://dx.doi.org/10.1155/2016/2879809.
7. World Union of Wound Healing Societies. Local management of diabetic foot ulcers. *Wound Int* 2016.
8. Wound International. International best practice guidelines: wound management in diabetic foot ulcers. *Wounds Int* 2013;**5**:27. Available from: www.woundsinternational.com.
9. Health Service Executive (HSE). *National best practice and evidence based guidelines for wound management*. Dublin: HSE; 2009.
10. Armstrong DG, Bohn G, Glat P, Kavros SJ, Kirsner R, Snyder R, et al. Expert recommendations for the use of hypochlorous solution: science and clinical application. *Ostomy Wound Manage* 2015;**61**:S2–19.
11. Yamaner C, Ayvaz M, Konak R, Tan N, Kosoglu I, Dimoglo A. Efficacy of neutralised electrolysed water and mild heat against foodborne pathogens isolated from ficus carica. *Ital J Food Sci* 2016;**28**:208–20.
12. Ignacio RMC, Joo K, Lee K. Clinical effect and mechanism of alkaline reduced water. *J Food Drug Anal* 2012;**20**:394–7.
13. Kumon K. What is functional water? *Artif Organs* 1997;**21**:2–4.
14. Rahman S, Khan I, Oh D. Electrolyzed water as a novel sanitizer in the food industry: current trends and future perspectives. *Compr Rev Food Sci Food Saf* 2016;**15**:471–90, http://dx.doi.org/10.1111/1541-4337.12200.
15. Pankey GA, Sabath LD. Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of Gram-positive bacterial infections. *Clin Infect Dis* 2004;**38**:864–70.
16. Al HAq MI, Sugiyama J, Isobe S. Applications of electrolyzed water in agriculture & food industries. *Food Sci Technol* 2005;**11**:135–50.
17. Len SV, Hung YC, Chung D, Anderson JL, Erickson MC, Morita K. Effects of storage conditions and ph on chlorine loss in electrolyzed oxidizing (EO) water. *J Agric Food Chem* 2002:209–12.
18. Huang Y, Hung Y, Hsu S, Huang Y. Application of electrolyzed water in the food industry. *Food Control* 2008;**19**:329–45, http://dx.doi.org/10.1016/j.foodcont.2007.08.012.
19. Park EJ, Alexander E, Taylor GA, Costa R, Kang DH. The decontaminative effects of acidic electrolyzed water for *Escherichia coli* O157:H7 *Salmonella typhimurium*, and *Listeria monocytogenes* on green onions and tomatoes with differing organic demands. *Food Microbiol* 2009;**26**:386–90, http://dx.doi.org/10.1016/j.fm.2008.10.013.
20. Liao LB, Chen WM, Xiao XM. The generation and inactivation mechanism of oxidation – reduction potential of electrolyzed oxidizing water. *J Food Eng* 2007;**78**:1326–32, http://dx.doi.org/10.1016/j.jfoodeng.2006.01.004.
21. Koseki S, Yoshida K, Isobe S, Itoh K. Decontamination of lettuce using acidic electrolyzed water. *J Food Prot* 2000;**64**:652–8.
22. Abbasi PA, Lazarovits G. Effect of acidic electrolyzed water on the viability of bacterial and fungal plant pathogens and on bacterial spot disease of tomato. *Can J Microbiol* 2006;**923**:915–23, http://dx.doi.org/10.1139/W06-048.
23. Issa-Zacharia A, Kamitani Y, Tiisekwa A, Morita K, Iwasaki K. In vitro inactivation of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* spp. using slightly acidic electrolyzed water. *J Biosci Bioeng* 2010;**110**:308–13, http://dx.doi.org/10.1016/j.jbiosc.2010.03.012.
24. Nan S, Li Y, Li B, Wang C, Cui X, Cao W. Effect of slightly acidic electrolyzed water for inactivating *Escherichia coli* O157: H7 and *Staphylococcus aureus* analyzed by transmission electron microscopy. *J Food Prot* 2010;**73**:2211–6.
25. Xuan X, Ding T, Li J, Ahn J, Zhao Y, Chen S, et al. Estimation of growth parameters of *Listeria monocytogenes* after sublethal heat and slightly acidic electrolyzed water (SAEW) treatment. *Food Control* 2017;**71**:17–25, http://dx.doi.org/10.1016/j.foodcont.2016.06.018.
26. Yang H, Swem B, Li Y. The effect of pH on inactivation of pathogenic bacteria on fresh-cut lettuce by dipping treatment with electrolyzed water. *J Food Sci* 2003;**68**:1013–7.
27. Sun J, Zhang S, Chen J, Han B. Efficacy of acidic and basic electrolyzed water in eradicating *Staphylococcus aureus* biofilm. *Can J Microbiol* 2012;**454**:448–54, http://dx.doi.org/10.1139/W2012-005.