International Journal of GEOMATE, Aug., 2020, Vol.19, Issue 72, pp. 211 - 216
ISSN: 2186-2982 (P), 2186-2990 (O), Japan, DOI: https://doi.org/10.21660/2020.72.5624
Special Issue on Science, Engineering and Environment

COMBINED EFFECTS OF ACIDIC ELECTROLYZED WATER AND ULTRASOUND TREATMENTS TO DECONTAMINATE FRESH TUMERIC

* Santirote Keatsirirote[1,2] Angkhana Chuajedton[4] Jamnong Uthaibutra[1,2,3] and Kanda Whangchai[1,2,3]

[1]Postharvest Technology Research Center, Faculty of Agriculture,
Chiang Mai University, Chiang Mai, Thailand
[2]Postharvest Technology Innovation Center, Office of the Higher Education Commission,
Bangkok, Thailand
[3]Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand
[4]Department of Biology, Faculty of Science, Lampang Rajabhat University, Lampang, Thailand

*Corresponding Author, Received: 30 June 2019, Revised: 04 March 2020, Accepted: 13 March 2020

ABSTRACT: The effect of electrolyzed oxidizing water (EO) and ultrasonic waves (US) on pathogenic control of fresh turmeric (*Curcuma longa* L.) were studied. Electrolyzed water was generated by electrolysis from 5% NaCl solution. The fresh turmeric samples were washed with EO at different concentrations (0, 100, 200, 300 and 400 mg/L residual chlorine). The percentage of reduction of total bacteria, yeast, mold and *Escherichia coli* was determined by compact dry plate. It was shown that EO at 300 mg/L significantly inhibited the cell growth. Then, the samples were washed with combination of EO and US for 10 minutes. It was shown that combination of EO and US significantly inhibited bacterial growth and EO 200 mg/L with US at 43 kHz had high value of log reduction when compared with other treatments. Therefore, combination of EO and US appeared to increasing pathogenic control of fresh turmeric.

Keywords: Electrolyzed oxidizing water, Pathogenic control, Turmeric, Ultrasonic wave

1. INTRODUCTION

Turmeric (*Curcuma longa* L.) is an edible and medicinal plant in the family Zingiberaceae, known as Indian saffron. Turmeric rhizome when dry and ground provides a yellow and flavorful powder, used for natural coloring agent in food, cosmetic and textile. Also flavoring compounds as insect repellent and medicine. Recently, it has been valued worldwide as a functional food, due to its health promoting properties. Turmeric has been used as antioxidant, digestive, anti-microbial, anti-inflammatory and anti-carcinogen [1].

Thailand is one of the major exporter countries for turmeric. [2,3]. However, the main problem of fresh turmeric is the microbial contamination during harvest [4]. Naruemon and Dariwan [5] reported that the contamination of turmeric was due to mold (40.45%) and *Escherichia coli* (62.34%). Postharvest disease control can be done with many methods, such as chemical disinfection, hot water treatment or washing with clean water. Washing fresh turmeric with hot water is recommended in many countries, such as India [6]. In Thailand, the harvested turmeric distributed in the markets is washed with clean water only. Normally, the fungi and bacteria are present in the packing-house and in the field after harvest. Moreira [7] reported that the symptoms of rot ginger (*Zingiber officinale* Roscoe) rhizomes in Brazil was due to from *Fusarium oxysporum* (74%). Recently, electrolyzed water (EO) has been reported to have strong anti-bacterial effects on most pathogenic bacteria [8]. EO is a powerful bactericidal agent and is applied in a wide range of fields, including aquaculture, agriculture and food industry [9] [10]. Buck [11] used EO to treat 22 fungal species and found that it significantly reduced growth of the thin-walled fungi (e.g., *Botrytis* and *Monilinia*) within 30 seconds. Additionally, it significantly reduces the growth of the thicker-walled, pigmented fungi, *Curvularia* and *Helminthosporium* within 2 minutes or less. In addition, Deza [12] found that tomato peel treated with EO resulted in a decline of bacteria such as *Escherichia coli* 0157:H7, *Salmonella enteritidis* and *Listeria monocytogenes*, without any effect on the environment. Hung [13] reported that EO treatment on strawberries and broccoli significantly reduced *E. coli* 0157:H7. Paola [14] found that washing lettuce with EO for 5 minutes significantly inhibited the growth of *L. monocytogenes*. Guentzel [15] also found that spraying EO at the surfaces of food service areas on spinach and lettuce reduced the growth of microbes. Whangchai [16] found that EO treatment on tangerine cv. Sai Nam Pung at a free chlorine concentration of 215 ppm for 120 and 240 seconds completely inhibited the growth and development of *Penicillium digitatum*. In addition, Whangchai [17] found that washing orange with EO and exposure to ozone gas for 2 hours per day significantly controlled *P. digitatum*. US has been

used in postharvest treatment to reduce decay incidence and maintain the quality of fruits and vegetables. The high power ultrasound wash water decontamination process in the fruit and vegetable industries is effective to decrease foodborne outbreaks [18]. Ultrasound treatment has been reported to be effective in food processing and preservation, such as clean surfaces, inactivation of microorganisms and enzymes, disruption of cells [19]. Thus, the objective of this research was to determine the effect of EO with US on pathogenic control of fresh turmeric.

2. MATERIALS AND METHODS

2.1 Plant materials

Fresh turmeric (*Curcuma Longa* L.) was collected from a commercial turmeric farm in Mae-Tang, Chiang Mai Province, Thailand and transported to the Postharvest Physiology Research Laboratory at Chiang Mai University within 3 hrs. Fresh turmeric rhizomes (50 gram) were sampled for each replicate. All the experiments were designed as a Completely Randomized Design (CRD) and each treatment was done in triplicate.

2.2 Preparation of electrolyzed water

EO was generated by electrolysis in a cell with positively and negatively charged titanium electrodes separated by a polypropylene membrane. The electrodes were subjected to a direct current of 8 volts. A 5% NaCl solution was added into the above system (Fig. 1). The pH of EO water was recorded with pH/ion meter. Free–chlorine concentration was determined by using N,N-diethyl-P-phenylene diamine (DPD) test and oxidation-reduction potential (ORP) was measured by pH/ ORP meter.

2.3 Preparation of ultrasonic wave

US devices with the input power of 3 watts and 2 frequencies of 43 and 1000 kHz, (Honda Electronics Company, Toyohashi, Aichi, Japan) were utilized for 3 replications. A polyethylene cylinder reactor, 10 cm in diameter, equipped with a transducer at the lower part was used.

2.4 Effect of electrolyzed water on microbial growth inhibition

Fresh turmeric (50g for each replication) was immersed in 450 ml of EO at different concentrations (0, 100, 200, 300 and 400 mg/L residual chlorine) for 10 minutes and then the samples were subjected to microbiological analysis by putting 25g fresh turmeric into 225ml sterile 0.1% peptone and homogenizing in a stomacher blender for 2 min and 30 second. Serial ten-fold dilution of the homogenate was done in 0.1% peptone. One milliliter of each dilution was dropped onto compact dry plate (R-Biopharm AG Company, Darmstadt, Germany). All the plates were incubated at 37°C for 24 hr for E. coli and total plate counts, and incubated at room temperature (28±2°C) for 72 hr for yeast and mold.

2.5 Effect of electrolyzed water and ultrasonic wave on microbial growth inhibition

Fresh turmeric (50g) was immersed in 450 ml of EO at different concentrations (0, 100 and 200 mg/L residual chlorine) combined with US at 43 and 1000 kHz for 10 minutes. The samples were also microbiologically analyzed. Fresh turmeric 25g from each treatment was put into 225ml sterile 0.1% peptone and homogenized in a stomacher blender for 2 min. and 30 second. Serial ten-fold dilution of the homogenate was done in 0.1% peptone. One milliliter of each dilution was dropped onto compact dry plate (R-Biopharm AG Company, Darmstadt, Germany) All the plates were incubated at 37°C for 24 hr for E. coli and total plate counts, and incubated at room temperature (28±2°C) for 72 hr for yeast and mold.

2.6 Statistical analysis

All the experiments were replicated three times and evaluated with regression procedure using SPSS version 17. Differences among treatments were performed using Duncan's Multiple Range test ($P > 0.05$).

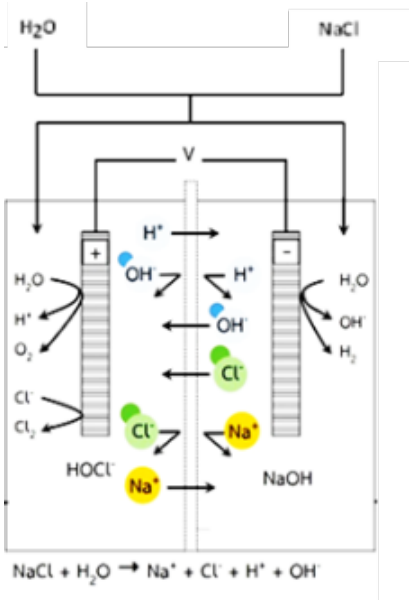

Fig. 1 Schematics of electrolyzed water generator. (Huang [20])

3. RESULTS AND DISCUSSION

3.1 Effect of EO on microbial growth inhibition

The reduction in microbial cells is shown in Fig. 2. EO significantly inhibited the cell growth at 100 mg/L and EO at 300 and 400 mg/L obtained the best result in the reduction of *E. coli* by 1.80 and 1.84 log CFU/g, respectively (Fig. 3). The total plate count, also showed that EO at 300 mg/L significantly inhibited the cell growth by 1.47 log CFU/g when compared with the control. EO was effective against bacteria. Tomás-Callejas [21] reported that washing fresh-cut baby mizuna leaves with EO showed an inhibitory effect on natural microflora. In another report, Issa-Zacharia [22] demonstrated that EO treatment significantly reduced the total aerobic mesophilic bacteria from Chinese celery, lettuce and daikon sprouts by ≥ 2.5 log CFU/g. *E.coli* and *Salmonella* spp. were also significantly reduced by ≥ 2.7 log CFU/g and ≥ 2.9 log CFU/g, respectively. EO has a strong oxidation potential and bactericidal effect that can be used as a disinfectant. Electrolyzed oxidizing water (pH < 2.7, ORP > 1100 mV) and the presence of hypochlorous acid were produced at the anode side. This solution has a strong oxidation potential in which oxidation-reduction potential (ORP) was measured for the oxidation reaction of all treatments with EO as represented in Table 1. The highest value of ORP occurred in EO treatment at 400 mg/L. The ORP showed more oxidation efficiency which promoted the rising of oxidation efficiency by the concentrations. Suslow [18] reported that an ORP had the activity to eliminate the pathogens that promoted the rising of ORP. An ORP at 650-700 mV reduced the sterilization time of *Escherichia coli*, *Salmonella* sp. and *Listeria* sp. when compared with the ORP lower than 485 mV.

3.2 Effect of electrolyzed water and ultrasonic wave on microbial growth inhibition

Combination of EO and US inhibited the bacterial growth. US significantly inhibited the cell growth at US 43 kHz and 1000 kHz and obtained the high value of log reduction of *E. coli* by 1.69 and 0.63 log CFU/g, respectively (Fig. 4). The total plate count, also showed that US at 43 kHz and 1000 kHz significantly inhibited the cell growth when compared with the control. Combination of EO and US significantly inhibited bacterial growth. Fig. 5. shows log reduction of *E. coli* after treatment with EO 0 +US 43 kHz, EO 100 +US 43 kHz, EO 200 +US 43 kHz, EO 300+US 43 kHz and EO 400+US 43 kHz by 1.19, 1.15, 1.00,0.45 and 0.54 log CFU/g, respectively. Meanwhile EO 200 +US 1000 kHz, EO 100 +US 1000 kHz, EO 200 +US 1000 kHz, EO 300 +US 1000 kHz and EO 400 +US 1000 kHz could inhibit the growth at 0.93, 0.35, 0.63,0.46 and 0.36 log CFU/g, respectively. Hung [13] reported that a combination of EO and US resulted in a greater reduction of the bacterial contamination of broccoli. For total plate count, EO 100 +US 43 kHz gave the best result in log reduction by 2.14 log CFU/g. For yeast & mold, EO 200 +US 43 kHz obtained the best result in log reduction by 1.27 log CFU/g. The efficiency of EO and US combined showed a significant enhanced control of microbial cells. US destroyed microorganism by the physical forces of cavitation which is the mechanical effect responsible for the destruction of fungal cells [23]. In the same way, Cao [24] used ultrasound treatment on strawberries at 0, 25, 28, 40 or 59 kHz at 20°C for 10 min, and then stored at 5°C for 8 days. Hypochlorous acid damages the microbial cell by oxidizing nucleic acids and proteins, causing lethal damage [25]. Scouten and Beuchat [26] reported that combined treatments of chemical, heat, and ultrasound were effective in killing *Salmonella* and *E. coli* O157:H7 on alfalfa seeds. The result of this research also suggested that the application of EO combined with US could pathogenically control fresh turmeric.

Table 1 Effect of electrolyzed water on pH, and ORP (mV)

Treatments	Parameters	
	pH	ORP (mV)
No washed (NW)	-	-
Distilled water (DW)	6.68	200
EO 100 mg/L	2.64	224
EO 200 mg/L	2.51	238
EO 300 mg/L	2.50	246
EO 400 mg/L	2.39	250

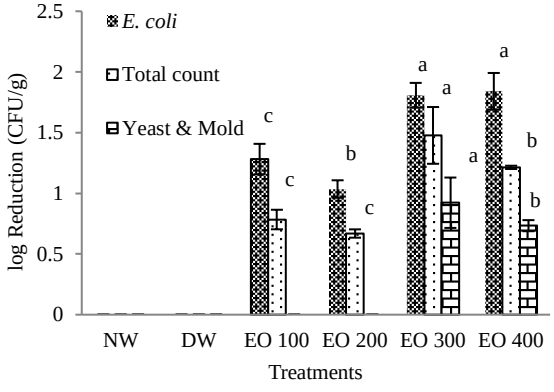

Fig. 2 Effect of electrolyzed water on microbial growth inhibition after incubation at 37 °C for 24 h. *Error bar represented standard deviations. Values with the same letter on the same color bars represented no significant differences (*P>0.05)

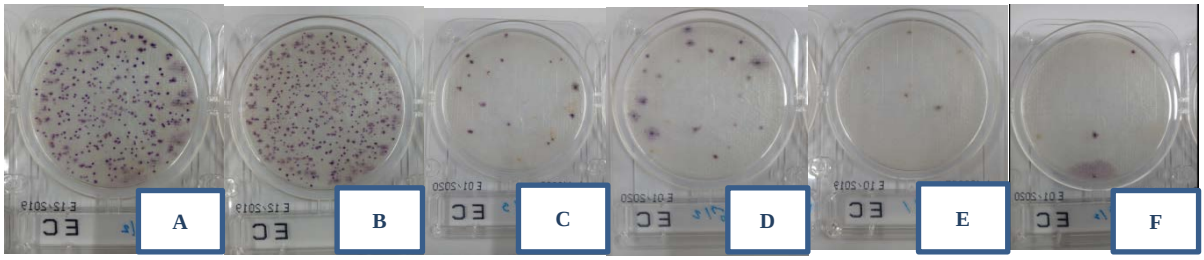

Fig. 3 Effect of electrolyzed water (A) control (no washed: NW) (B) distilled water: DW (C) EO 100 ppm (D) EO 200 ppm (E) EO 300 ppm (F) EO 400 ppm on growth inhibition of *E. coli* after incubation at 37 °C for 24 hr.

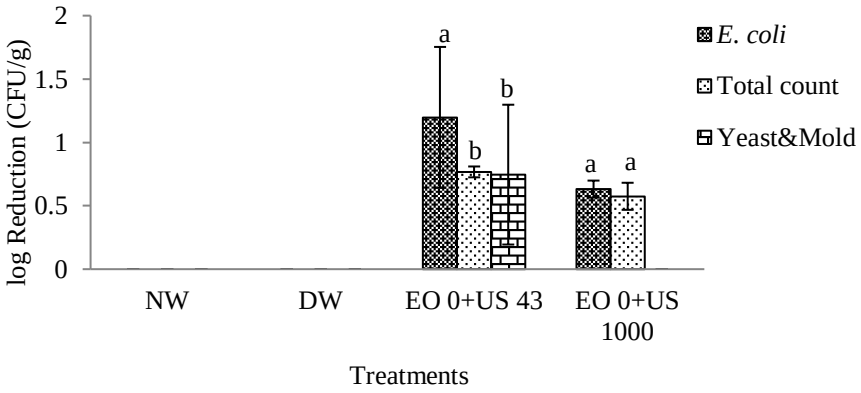

Fig. 4 Effect of ultrasonic wave on microbial growth inhibition after incubation at 37 °C for 24 h. *Error bar represented standard deviations. Values with the same letter on the same color bars represented no significant differences (*P>0.05)

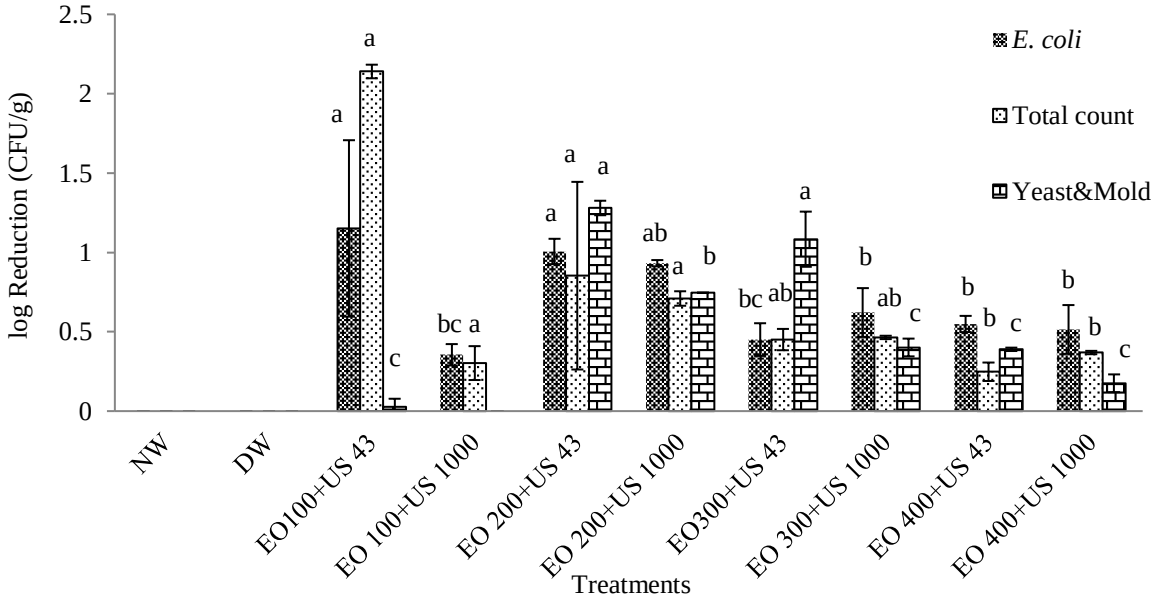

Fig. 5 Effect of electrolyzed water and ultrasonic wave on microbial growth inhibition after incubation at 37 °C for 24 h. *Error bar represented standard deviations. Values with the same letter on the same color bars represented no significant differences (*P>0.05)

4. CONCLUSION

EO significantly inhibited the cell growth and the application of EO combined with US (EO 200 mg/L with US at 43 kHz) gave the best inhibition of microbial cells (*E. coli*, total plate count, and yeast and mold). Therefore, EO and US could enhance pathogenic control of fresh turmeric and applied in the agriculture and food industry.

5. ACKNOWLEDGMENTS

This work was supported by Postharvest Technology Research Center, Faculty of Agriculture, Chiang Mai University, Chiang Mai and Postharvest technology Innovation Center, Commission on Higher Education, Bangkok, Thailand.

6. REFERENCES

[1] Bambirra M. L. A., Junqueira R. G. and Gloria B. A. Influence of postharvest processing conditions on yield and quality of ground turmeric (Curcuma longa L.). Brazillian Archieves of Biology and technology, Vol. 45, Issue 4, 2002, pp.423-429.

[2] TNAU Agritech Portal.. Tumeric (Online). 2015.

[3] Selvan S. Pre and postharvest processing of turmeric, 2016.

[4] Monton C. Microbial contamination of turmeric capsules produced at a secondary government hospital in the northeastern Thailand. Thai Journal of Pharmaceutical Science, Vol. 40, Supplement Issue, 2016, pp. 143-145.

[5] Naruemon C. and Dariwan S. Microorganism contamination and standard of supplement products consumed in household case study: Municipal of Thapra, Muang District, Khon Kean Province. KKU Research Journal, Vol. 14, Issue 3, 2014, pp. 81-91.

[6] FAO. Turmeric post-harvest operation. Food and Agriculture Organization of the United Nation (FAO.), AGST, 2004, pp.20.

[7] Moreira S. I., Dutra D.C., Rodrigues A. C., Oliveira J. R., Dhingra O. D. and Pereira O. L. Fungi and bacteria associated with post-harvest rot of ginger rhizomes in Espírito Santo, Brazil. Tropical Plant Pathology, Vol. 38, Issue 3, 2013, pp.218-226.

[8] Al – Haq M. I., Seo Y., Oshita S. and Oshita Y. Disinfection effects of electrolyzed oxidizing water on suppressing fruit rot of pear caused by Botryosphaeria berengeriana. Food Research International, Vol. 35, 2002, pp. 657–664.

[9] Whangchai N., Nomura N. and Matsumura M. Factors affecting phytoplankton removal by electro- oxidation of artificial seawater. Chiangmai Journal of Science, Vol. 30, 2003, pp.255 – 259.

[10] Kim C., Hung Y.C. and Brackett R.E. Efficacy of electrolyzed oxidizing (EO) and chemically modified water on different types of foodborne pathogens. International Journal of Food Microbiology, Vol. 61, 2000, pp.199–207.

[11] Buck J.W., Van Iersel M.W., Oetting R.D. and Hung C.Y. In vitro fungicidal activity of acidic electrolyzed oxidizing water. Plant Disease, Vol. 86, 2002, pp. 278-281.

[12] Deza M.A., Araujo M. and Garido J. M. Inactivation of Escherichia coli O157:H7, Salmonella enteritidis and Listeria monocytogenes on the surface of tomatoes by neutral electrolyzed water. The Society for Applied Microbiology, Vol. 37, 2003, pp. 482-487.

[13] Hung Y. C., Tilly P. and Kim C. Efficacy of electrolyzed oxidizing (EO) water and chlorinated water for inactivation of Escherichia coli 0157:H7 on strawberries and broccoli. Journal of Food Quality, Vol. 33, 2010, pp. 559-577.

[14] Paola C. L., Vivian R. C., Mercado M., Deaz M., and Carrascal A. K. Effectiveness of electrolyzed oxidizing water for inactivating Listeria monocytogenes in lettuce. Universitas Scientiarum, Vol. 10, 2005, pp.97-108.

[15] Guentzel J. L., Liang L. K., Callan M. A., Emmons S. A. and Dunham V. L. Reduction of bacteria on spinach, lettuce, and surfaces in food service areas using neutral electrolyzed oxidizing water. Food Microbiology, Vol. 25, Issue 1, 2008, pp. 36-41.

[16] Whangchai K., Saengnil K., Singkamanee C. and Uthaibutra J. Use of Electrolyzed oxidizing water to control postharvest disease during storage of tangerine cv. Sai Nam Pung. Acta Horticultrae, Vol. 837, 2009, pp. 211-215.

[17] Whangchai K., Saengnil K., Singkamanee C. and Uthaibutra J. Effect of electrolyzed oxidizing water and continuous ozone exposure on the control of Penicillium digitatum on tangerine cv. Sai Nam Pung during storage. Crop Protection, Vol. 29, 2010, pp.386-389.

[18] Suslow T.V., Oxidation-reduction potential (ORP) for water disinfection monitoring, control, and documentation. University of California. Division of Agriculture and Natural Resources, Publication, 2004, pp.8149.

[19] Knorr D., Zenker M., Heinz V. and Lee D.U., Applications and potential of ultrasonic in food processing. Trends in Food Science and Technology, Vol. 15, 2004, pp. 261–266.

[20] Huang Y.R., Hung Y.C., Hsu S.Y. Huang Y.W. and Hwang D.F. Application of electrolyzed

water in the food industry. Food Control, Vol. 19, 2007, pp. 329–345.

[21] Tomás-Callejas A., Martinez-Hernandez G.B., Artés F., Artés-Hernández F. and Trevor V.S. Neutral and acidic electrolyzed water as emergent sanitizers for fresh-cut mizuna baby leaves. Postharvest Biology and Technology, Vol. 55, 2011, pp.114 -120.

[22] Issa-Zacharia A., Kamitani Y., Miwa N., Muhimbula H. and Iwasaki K. Application of slightly acidic electrolyzed water as a potential non-thermal food sanitizer for decontamination of fresh ready-to-eat vegetables and sprouts. Food Control, Vol. 22, 2010, pp. 601-607.

[23] Piyasena P., Mohareb E. and McKellar R. C. Inactivation of microbes using ultrasound: a review. International Journal of Food Microbiology, Vol. 87, Issue 3, 2003, pp. 207-216.

[24] Cao S., Hu Z. and Pang B. Optimization of postharvest ultrasonic treatment of strawberry fruit. Postharvest Biology and Technology, Vol. 55, 2010a, pp. 150 – 153.

[25] Acher A., Fisher E., Turnheim R. and Manor,Y. Ecologically friendly wastewater disinfection techniques. Water Research, Vol. 31, 1997, pp. 1398–1404.

[26] Scouten A. J. and Beuchat L.R. Combined effects of chemical, heat and ultrasound treatments to kill Salmonella and Escherichia coli O157:H7 on alfalfa seeds. Journal of Applied Microbiology, Vol. 92, 2002, pp.668–674.