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Application of electrolyzed oxidizing water on the reduction of bacterial contamination for seafood

Yu-Ru Huang [a], Hung-Sheng Hsieh [a], Shin-Yuan Lin [a], Shin-Jung Lin [b], Yen-Con Hung [c], Deng-Fwu Hwang [a,*]

[a] Department of Food Science, National Taiwan Ocean University, Keelung 20224, Taiwan, ROC
[b] Department of Food Science and Technology, Ching-Kuo Institute of Management and Health, Keelung 20224, Taiwan, ROC
[c] Department of Food Science and Technology, University of Georgia, Griffin, GA 30223-1797, USA

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Abstract

For reducing bacterial contamination, electrolyzed oxidizing water (EO water) has been used to reduce microbial population on seafood and platform of fish retailer. The specimens of tilapia were inoculated with *Escherichia coli* and *Vibrio parahaemolyticus*, and then soaked into EO water for up to 10 min. EO water achieved additional 0.7 log CFU/cm^2 reduction than tap water on *E. coli* after 1 min treatment and additional treatment time did not achieved additional reduction. EO water treatment also reduced *V. parahaemolyticus*, by 1.5 log CFU/cm^2 after 5 min treatment and achieved 2.6 log CFU/cm^2 reduction after 10 min. The pathogenic bacteria were not detected in EO water after soaking treatment. In addition, EO water could effectively disinfect the platform of fish retailer in traditional markets and fish markets.
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Keywords: Electrolyzed oxidizing water; Tilapia; Oyster; *Escherichia coli*; *Vibrio parahaemolyticus*

1. Introduction

Food safety is an increasingly important public health issue. Governments all over the world are intensifying their efforts to improve food safety. These efforts are in response to an increasing number of food safety problems and rising consumer concerns. *Escherichia coli* strains that are pathogenic for humans and cause diarrheal illness may be categorized into specific groups based on virulence properties, mechanisms of pathogenicity, and clinical syndromes. *E. coli* O157:H7 was first identified as human food-borne pathogens in 1982, when *E. coli* strains of a previously uncommon serotype, O157:H7, were implicated in two outbreaks of hemorrhagic colitis (bloody diarrhea) in the United States (US) (Doyle, 1991). Since then, outbreaks of this pathogen have become a serious public health problem throughout many regions of the world (Clarke, Haigh, Freestone, & Williams, 2002; Schlundt, 2001). *Vibrio parahaemolyticus* is a prevalent food-borne pathogen in many Asian countries where marine foods are frequently consumed (Joseph, Colwell, & Kaper, 1983). It is an important food-poisoning pathogen in coastal countries, especially in Japan and Taiwan. In Taiwan, this pathogen accounts for more than half of the bacterial food-poisoning outbreaks occurred annually (Wang, Ho, Tsai, & Pan, 1996).

One way to reduce the incidence of food-borne disease is by providing a safe food supply. Electrolyzed oxidizing water (EO water) has been reported to possess antimicrobial activity on a wide variety of microorganisms including *Pseudomonas aeruginosa*, *Staphylococcus*

* Corresponding author. Tel.: +886 2 24622192x5103; fax: +886 2 24626602.
E-mail address: dfhwang@mail.ntou.edu.tw (D.-F. Hwang).

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aureus, *S. epidermidis*, *E. coli* O157:H7, *Salmonella enteritidis*, *S. typhimurium*, *Bacillus cereus*, *Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Campylobacter jejuni*, *Enterobacter aerogenes* and others (Fabrizio & Cutter, 2003; Horiba et al., 1999; Iwasawa & Nakamura, 1993; Kim, Hung, & Brachett, 2000a, 2000b; Kim, Hung, Brachett, & Frank, 2001; Kiura et al., 2002; Park & Beuchat, 1999; Park, Hung, & Brackett, 2002a; Venkitanarayanan, Ezeike, Hung, & Doyle, 1999b). Researchers also confirmed EO water to be effective against blood borne pathogenic viruses including hepatitis B virus (HBV) and human immunodeficiency virus (HIV) (Morita et al., 2000; Tagawa et al., 2000).

EO water is produced when dilute salt water is put through an electric current in a sealed chamber. Two types of water are produced from the process. The first stream is acidic, capable of killing harmful microorganisms. The second contains alkaline water, which can be used to remove dirt and grease from items such as cutting boards and other kitchen utensils. Several studies have shown that EO water is capable of reducing pathogens and/or spoilage organisms attached to cutting boards (Venkitanarayanan, Ezeike, Hung, & Doyle, 1999a), poultry carcasses (Fabrizio, Sharma, Demirci, & Cutter, 2002; Park et al., 2002a), eggs (Russell, 2003), lettuce (Izumi, 1999; Koseki & Itoh, 2001; Koseki, Fujiwara, & Itoh, 2002; Koseki, Yoshida, Isobe, & Itoh, 2001; Koseki, Yoshida, Kamitani, & Itoh, 2003; Park, Hung, Doyle, Ezeike, & Kim, 2001), alfalfa seeds, sprouts (Kim, Hung, Brackett, & Lin, 2003; Sharma & Demirci, 2003), pears (Al-Haq, Seo, Oshita, & Kawagoe, 2002a), apples (Colgan & Johnson, 1998; Okull & Laborde, 2004), peaches (Al-Haq, Seo, Oshita, & Kawagoe, 2002b), tomatoes (Bari, Sabina, Isobe, Uemura, & Isshiki, 2003) and food processing equipments (Blackman & Frank, 1996; Park, Hung, & Kim, 2002b). EO water has also inactivated staphylococcal enterotoxin-A and aflatoxin (Suzuki, Itakura, Watanabe, & Ohta, 2002a, 2002b).

Disinfectant effect of electrolyzed salt water on fish pathogenic bacteria and viruses was evaluated. Researchers suggested the feasibility of seawater electrolysis as an efficient and comparatively low-cost alternative method for the control of opportunistic pathogens both in hatchery culture systems and for massive culture of microalgae (Kasai, Ishikawa, Hori, Watanabe, & Yoshimizu, 2000; Nakajima et al., 2004). In fact most of the published literature concentrates on the studying of use of EO water as a disinfectant conventional treatment. Using EO water to treat raw salmon, and achieved up to a 1.07 log CFU/g (91.1%) reduction in *E. coli* O157:H7 and 1.12 log CFU/g (92.3%) reduction in *L. monocytogenes* (Ozer & Demirci, in press). The purpose of this research was to determine the effect of EO water for inactivation of *V. parahaemolyticus* and *E. coli* on tilapia surfaces and disinfecting platform bacteria of fish retailer in traditional markets and fish markets.

2. Materials and methods

2.1. Bacterial cultures

A five-strain mixture of *E. coli* (BCRC 10675, urine isolate; 16070, clinical isolate; 15970, human stool from outbreak of hemorragic colitis isolate and strain of *E. coli* O157:H7; 15376, human feces isolate; and 15371, infant diarrheatic stool isolate) and a five-strain mixture of *V. parahaemolyticus* (BCRC 13023, case of food poisoning in Taiwan; 12972, shrimp isolate; 12964, oyster isolate; 12966, clam isolate; and 12962, crab isolate) were used for the study. All of the strains were stock cultures obtained from Bioresources Collection and Research Center (BCRC, Food Industry Research and Development Institute, Hsinchu, Taiwan). Each bacterial strain of *V. parahaemolyticus* was cultured individually in 10 ml sterile tryptic soy broth (TSB, Merck) supplemented with 3% NaCl for 24 h at 37 °C as a stock culture for tests. Each bacterial strain of *E. coli* was cultured individually in 10 ml sterile TSB at 37 °C for 24 h. Each strain was transferred three times to TSB by loop inoculate at successive 24 h intervals before use as inoculum on tilapia. Following incubation, the bacterial cells were sedimented by centrifugation (2000*g*, 22 °C for 15 min) and its pellet was suspended in 2 ml of 0.1% sterile peptone water (pH 7.1). For each pathogen, equal portion (2 ml each) from each of the 5 strains was combined to make the inoculum containing approximately equal numbers of cells of each strain of *V. parahaemolyticus* (10^8 CFU/ml) and *E. coli* (10^8 CFU/ml), respectively.

2.2. Preparation of electrolyzed oxidizing water

Electrolyzed oxidizing water was generated with a ROX-20TA EO water generator (Hoshizaki Electric Company Ltd., Toyoake, Aichi, Japan). A continuous supply of deionized water and 12% sodium chloride solution at room temperature was pumped into the equipment. Acidic electrolyzed solution is produced from the anode side that has low pH (<2.8), high oxidation reduction potential (ORP, around 1100 mV) and chlorine which concentration is depending on the EO water machine setting. Generally, the EO water generator electrolyzed at 19.9 A and flow rate at around 2000 ml/min, the generated acidic EO water contains 100 ppm available chlorine. Electrolysis efficiency was significantly increased by decreases in water flow rate. In this study the EO water generator electrolyzed at amperage setting of 7, 14, 19.9 A and flow rate at

around 500 ml/min to produce acidic EO water containing 50, 100 and 200 ppm available chlorine, respectively. The generator was allowed to run for about 15 min before collecting water for the treatment so that the amperage level, as shown by the display indicator, was stabilized to the set value. The EO water was collected from the appropriate outlet in sterile containers and was used within 2–3 h for the microbial study. Samples used for the determination of pH, oxidation reduction potential and free-chlorine concentration were also collected at the same time.

2.3. Determination of pH, ORP, and free chlorine

The pH values and ORPs of the tested solution were measured with a pH meter (Mettler Delta 320; Instruments Co., Ltd., Taiwan) with electrodes (Type: GST-5211C 205F; TOA Electronics Ltd., Japan for pH and Type: Hi3131B; Hanna Instruments Co., Ltd., Portugal for ORP). The free chlorine contents of the EO water was measured using a of Hach pocket colorimeter^{TM} analysis system (Hach CO., Loveland, Co) as described by the manufacturer. Briefly, a sample diluted to 0–2 mg/l with distilled water, filled a clean sample cell to the 10 ml mark with the diluted sample. A DPD Free Chlorine Powder Pillow was added to the sample cell. Cap and shake the cell for 20 s. Then, place the sample cell containing the diluted sample into the cell holder and cover with the instrument cap. The display will indicate the free chlorine concentration in milligrams per liter (mg/l).

2.4. Preparation and inoculation of tilapia sample

Tilapias (approximately 600 $\pm$ 20 g per sample) were purchased from a local grocery store and stored at 4 °C before use. For inoculation, each tilapia was checked to ensure it is intact and has complete coverage of skin on each tilapia. Each sample was then inoculated with *E. coli* or *V. parahaemolyticus* by gently spreading 3 ml of inoculum onto the skin surface and air-dried under a biosafety hood for 20 min at room temperature (23 $\pm$ 2 °C) to allow for bacterial attachment. The level of *E. coli* and *V. parahaemolyticus* in tilapia samples was ensured to be of approximately 7.0 log CFU/cm^2 and 5.5 log CFU/cm^2, respectively.

2.5. Treatment and bacteriological analysis of tilapia samples

Inoculated tilapias (600 $\pm$ 20 g) were individually immersed in 5 volumes each of EO water at a temperature of 23 $\pm$ 2 °C for 1, 5 and 10 min, respectively with agitation (150 rpm) on a platform shaker (Model No. TS-500, Yihder Instruments CO., Ltd.). Simultaneously, inoculated tilapias were also immersed in 5 volumes each of tap water (control) under the same conditions and durations as described above. Immediately after treatment, all samples were removed from treatment solutions and the surviving population of the pathogen on each tilapia and in the treatment solution was determined.

Bacterial counts on tilapias were determined by swabbing three areas of skin (5 by 5 cm each) individually with sterile swabs. Each swab was transferred to a test tube containing 10 ml of sterile Butterfield's phosphate buffer and vortexed vigorously to dispense bacteria into the buffer. Surviving bacteria in the solution after treatment was determined through serially diluting in 9 ml of sterile 0.1% Butterfield's phosphate buffer and then directing plating 0.1 ml of each dilution in duplicate on *Vibrio* selective agar (thiosulfate-citrate-bile salts-sucrose, TCBS, Merck) or *E. coli* selective agar (eosin methylene blue agar, EMB, Merck). Colonies were counted after incubation at 37 °C for 48 h.

Enrichment was performed to detect the presence of low number of survivors that would not be detected by directed plating by transferring 1 ml of each sample solution into 9 ml of sterile TSB supplemented with 3% NaCl and TSB for *V. parahaemolyticus* and *E. coli*, respectively, and incubated at 37 °C for 24 h. Furthermore, susceptive colonies of *V. parahaemolyticus* and *E. coli* from enrichment were further enumerated on TCBS and EMB agar at 37 °C for 48 h, respectively. Three independent replicate trials were conducted for each treatment solution.

2.6. Treatment and bacteriological analysis of platform of fish retailer in traditional markets and fish markets

The platform surface (100 $\times$ 150 cm) of fish retailers in traditional markets was constructed by ceramic tile (5 $\times$ 5 cm). This platform surface is usually cleaned with tap water at the end of the day. This platform surface at fish markets was constructed by cement mortar. This surface is usually cleaned by seawater at the end of the day. The treatment solution 10 ml was poured onto the platform at a temperature of 23 $\pm$ 2 °C for 1, 5 and 10 min, respectively. Treatment solutions included EO water containing 50, 100 and 200 ppm available chlorine, sterile deionized water, tap water and sea water.

After treatment, the total bacterial counts and *E. coli* on the platform were determined by swabbing three areas of the treated surface (5 by 5 cm each) with individual sterile swabs. Each swab was then transferred to a test tube containing 10 ml of sterile Butterfield's phosphate buffer and vortexed vigorously to dispense bacteria into the buffer. The phosphate buffer solution was then serially diluted (1:10) in sterile 0.1% Butterfield's phosphate buffer, and appropriate diluents were surface plated in duplicate on plate count agar (PCA,

Merck) and Coliform Petrifilm (3M Health Care Co., ST. Paul, Minn, USA). Colonies were counter after incubation at 37 °C for 24 h. Enrichment was performed to detect the presence of lower numbers of survivors that would not be detected by direct plating as described above. The experiment was replicated three times.

2.7. Statistical analysis

Each experiment was replicated three times. Data were analyzed using the General Linear Models procedure of SAS (Statistical Analysis Systems Institute, Cary, N.C.). Significant differences ($p \leqslant 0.05$) between mean values were determined using Duncan's multiple range test.

3. Results and discussion

3.1. Effect of EO water treatment on inactivation of E. coli and V. parahaemolyticus on tilapia

In this experiment, the initial pH, ORP and free chlorine concentration of tap water were 6.89 ± 0.05, 632 ± 8 mV, and 0.30 ± 0.02 ppm, respectively. Room-temperature EO water generated at a setting of 14 amperage and 10 voltage had initial pH, ORP and free chlorine concentration of 2.47 ± 0.02, 1159 ± 4 mV, and 120 ± 4 ppm, respectively. The initial population of each pathogen of *E. coli* and *V. parahaemolyticus* were 10^8 CFU/ml, respectively. The mean population of *E. coli* recovered from the tilapia after 20 min of drying was about 6.9 log CFU/cm^2. Research in the literature has shown that inactivation of bacteria on different food-processing surfaces with EO water treatment was enhanced with agitation. This is because the cells removed from the surface during agitation were immediately inactivated in EO water and agitation facilitates the penetration of EO water into the remaining cell on the test surfaces, or the well mixed EO water resulting from agitation allows chlorine to react with cells more efficiently (Park et al., 2002a). Thus treatment with agitation (150 rpm) was used this study.

Results presented in Table 1 indicated that inoculated tilapia soaking in EO water for 5 and 10 min has achieved additional reduction of *E. coli* population than tap water by 0.58 and 0.76 log CFU/cm^2, respectively. However, these results were not significantly different than the 1 min treatment (0.65 log CFU/cm^2 reduction).

The initial population of *V. parahaemolyticus* inoculated on tilapia was about 5.5–5.7 log CFU/cm^2. There was no significant difference between 1 min EO water and tap water treatment for reducing the *Vibrio* population on tilapia. Increased the treatment time to 5 min, EO water achieved 1.49 log CFU/cm^2 more reduction on *Vibrio* than tap water. Further increased the treatment time to 10 min, the population of *V. parahaemolyticus* on tilapia was reduced 2.61 log CFU/cm^2 when compared with tap water treatment. For the current study, detectable pathogens were still found on the tilapia after EO water treatment. Venkitanarayanan et al. (1999a) reported that EO water is highly effective in killing pathogen in pure culture. However, EO water is less effective for inactivating pathogens on tilapia and this may be due to the bactericidal activity of the EO water deteriorated in the presence of organic materials, which include amino acids and proteins (Oomori, Oka, Inuta, & Arata, 2000). Free chlorine in the EO water will react with organic materials and become combined available chlorine. Combined available chlorine has much lower bactericidal activity than the free form (White, 1992). Besides, bacterial cells were attached to a water-skin interface and further entrapped in crevices and pores and hence cannot be reached by EO water. Results of this study demonstrated that immersion of tilapia in EO water achieved 0.76 and 2.61 log CFU/cm^2 reduction

Table 1
Inactivation of *Escherichia coli* and *Vibrio parahaemolyticus* on tilapia by electrolyzed oxidizing water (EO water)

Bacterial species	Time (min)	Surviving population on tilapia (log CFU/cm^2)		Surviving population in soaking water (log CFU/ml)	
		Tap water[1]	EO water[2]	Tap water	EO water
E. coli	0	$6.93 \pm 0.15^{a,3}$	6.99 ± 0.32^a	$<1.0^b$	ND[4]
	1	7.19 ± 0.38^a	6.54 ± 0.37^a	5.73 ± 0.15^a	ND
	5	6.40 ± 0.51^a	5.82 ± 0.18^b	5.90 ± 0.27^a	ND
	10	6.07 ± 0.03^b	5.31 ± 0.12^c	6.07 ± 0.30^a	ND
V. parahaemolyticus	0	5.51 ± 0.08^a	5.69 ± 0.44^a	$<1.0^b$	ND[4]
	1	4.50 ± 0.02^b	4.55 ± 0.25^b	4.20 ± 0.13^a	ND
	5	4.60 ± 0.18^b	3.11 ± 0.18^c	3.98 ± 0.21^a	ND
	10	4.46 ± 0.03^b	1.85 ± 0.27^d	4.18 ± 0.05^a	ND

[1] Tap water, pH = 6.89 ± 0.05, ORP = 632 ± 8 mV, free chlorine concentration = 0.30 ± 0.02 ppm.
[2] EO water, pH = 2.47 ± 0.02, ORP = 1159 ± 4 mV, free chlorine concentration = 120 ± 4 ppm.
[3] Values in the same column with different superscript are significant difference ($P < 0.05$).
[4] Negative by an enrichment procedure and no detectable survivors by a direct plating procedure.

Table 2
Comparison on different concentration of electrolyzed oxidizing water to improve the hygienic quality of platform of fish retailer in traditional market

Treatment	Total bacteria count (log CFU/cm^2)				*E. coli*		pH	ORP (mv)	Free chlorine (ppm)
	0 min	1 min	5 min	10 min	0 min	5 min			
DH$_2$O[2]	4.86 ± 0.02[a,1]	3.83 ± 0.27[b]	3.83 ± 0.06[b]	3.35 ± 0.23[c]	–[5]	–	5.36 ± 0.12	405 ± 10	0
EO water (200 mg/l)	5.05 ± 0.07[a]	1.44 ± 0.91[b]	<1.0[c,3]	ND[c,4]	–	–	2.04 ± 0.03	1187 ± 1	210 ± 6
EO water (100 mg/l)	5.02 ± 0.76[a]	1.23 ± 0.11[b]	<1.0[c]	ND[c]	–	–	2.36 ± 0.04	1180 ± 3	100 ± 14
EO water (50 mg/l)	4.35 ± 0.37[a]	1.98 ± 0.28[b]	1.56 ± 0.64[b]	1.76 ± 0.81[b]	–	–	2.64 ± 0.07	1156 ± 5	55 ± 3

[1] Values in the same row with different superscript are significant difference ($P < 0.05$).
[2] DH$_2$O, sterile deionized water.
[3] Positive by an enrichment procedure and not detectable by a direct plating procedure.
[4] Negative by an enrichment procedure and no detectable survivors by a direct plating procedure.
[5] Negative by an enrichment procedure and no detectable survivors by a direct plating procedure.

on *E. coli* and *V. parahaemolyticus*, respectively and is an effective method for inactivating food-borne pathogens on tilapia surface.

Further, *E. coli* and *V. parahaemolyticus* were not detected in EO water after soaking treatment (Table 1), whereas the pathogens survived in the tap water used for soaking the tilapias, thus representing a potential source of cross-contamination or recontamination in case the same water is used for soaking or washing seafood. Similar results were reported by Park et al. (2001) and Park et al. (2002a). This demonstrated that EO water was very effective not only in reducing the population of *E. coli* and *V. parahaemolyticus* on treated samples, but also could prevent cross-contamination of processing environments.

Su and Morrissey (2003) studied the treatment of acidified sodium chlorite (50 ppm) for the skin of whole salmon and fillets and found 1 min washing treatment (10 liters per fish) reduced TPCs by 0.43 and 0.31 log CFU/cm^2, respectively. Ozer and Demirci (in press) also found that EO water has a potential to be used for decontamination of pathogens on raw salmon.

3.2. Efficacy of EO water in treating platform of fish retailer in traditional markets and fish markets

The initial bacterial counts on ceramic tiles for all trials ranged from 4.35 to 5.05 log CFU/cm^2 even though sellers had cleaned the platform surface before each measurement. On the platform surface of fish retailers, *E. coli* was not detected (Table 2). Statistically significant reductions in bacterial counts on platform of fish retailer in traditional market were observed in the EO water treatment containing 50, 100 and 200 ppm available chlorine for 1 min (Table 2). Increasing treatment time to 10 min with EO water containing 100 and 200 ppm available chlorine resulted in complete elimination of bacteria on the platform. Increased treatment time beyond 1 min for EO water containing 50 ppm chlorine achieved no additional reduction on bacterial counts. However, only 1 log CFU/cm^2 reduction on bacterial counts were observed in the sterile deionized water when compared with control (0 min of deionized water) regardless of treatment time.

The initial bacterial counts on platform surface from fish markets ranged from 4.37 to 5.66 log CFU/cm^2 for all trials. Our data showed that seawater was not effective for disinfecting the platform (Table 3). Similar results were observed in trials involving tap water (Table 3). EO water containing 50 ppm chlorine reduced the bacterial counts on platform in fish market for about 1.5 log CFU/cm^2. However, treatment time beyond 1 min had no additional effect. The total count of platform surface of traditional market was reduced by more than 3 log CFU/cm^2 when 10 min treatment of EO water containing 100 ppm chlorine was used (Table 3). Further increase the available chlorine concentration in EO water to 200 ppm reduced the total bacterial

Table 3
Comparison on different concentration of electrolyzed oxidizing water to improve the hygienic quality of platform of fish retailer in fish markets

Treatment	Total bacteria count (log CFU/cm^2)				*E. coli*		pH	ORP (mv)	Free chlorine (ppm)
	0 min	1 min	5 min	10 min	0 min	5 min			
Tap water	4.71 ± 0.23[a,1]	4.39 ± 0.28[a]	4.76 ± 0.15[a]	4.70 ± 0.15[a]	+	+	6.02 ± 0.01	707 ± 3	0.2 ± 0.02
Sea water	5.66 ± 0.23[a]	5.11 ± 0.83[a]	5.02 ± 0.64[a]	5.23 ± 0.28[a]	+	+	7.36 ± 0.02	293 ± 5	0
EO water (200 mg/l)	4.37 ± 0.03[a]	<1.0[a,2]	< 1.0[b]	< 1.0[b]	+	–[3]	2.24 ± 0.01	1145 ± 5	200 ± 5
EO water (100 mg/l)	5.17 ± 0.04[a]	4.07 ± 0.44[b]	2.74 ± 0.28[c]	2.12 ± 0.58[c]	+	–	2.55 ± 0.05	1120 ± 3	100 ± 4
EO water (50 mg/l)	4.72 ± 0.16[a]	3.32 ± 0.63[b]	3.27 ± 0.57[b]	3.53 ± 0.38[b]	+	–	2.70 ± 0.03	1090 ± 3	50 ± 2

[1] Values in the same row with different superscript are significant difference ($P < 0.05$).
[2] Positive by an enrichment procedure and no detectable by a direct plating procedure.
[3] Negative by an enrichment procedure and no detectable survivors by a direct plating procedure.

count to undetectable level for just 1 min treatment (Table 3).

In conclusion, our study demonstrates that EO water is very effective as a sanitizer for cleaning fish contacting surface of traditional market and fish market to prevent the fish and shellfish from secondary pollution of bacteria.

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