

Efficacy of Electrolyzed – Oxidizing Water for Inactivating *Escherichia coli* Inoculated on Holy Basil

Jantima Japakaset*, Siriporn Stonsaovapak, Pornthip Charoenthamawat
and Wanchai Panthavee

ABSTRACT

Electrolyzed – oxidizing (EO) water is a relatively new method that has been utilized for killing pathogens in agriculture, medical sterilization and food sanitation. This water is generated by passing sodium chloride solution through EO water generator. In this study, the EO water was used to treat holy basil inoculated with *Escherichia coli*. The initial pH and oxidation – reduction potential (ORP) of EO water were 2.09 and 1200 mV, respectively. The treatments changed ORP to 800, 950 and 1100 mV. The contact times were 10, 30 and 60 min. In pure culture, *E. coli* viable counts in the sample treated with EO water were reduced to undetectable levels at all ORP and times. However no reduction in *E. coli* counts was achieved in the control sample (treated with deionized water). The initial population of *E. coli* was about $8.5 \log_{10}$ CFU / ml which was inoculated on 5 g of holy basil. Results showed that the treatment treated with EO water was reduced about $2 \log_{10}$ CFU / ml in ORP 800 and 950 mV, $4 \log_{10}$ CFU / ml in ORP 1100 mV for 10 min. When the contact time increased to 30 min, the reduction of *E. coli* count was about $3 \log_{10}$ CFU / ml in ORP 950 mV and $5 \log_{10}$ CFU / ml in ORP 1100 mV. But the reduction was not different from 10 min when treated with ORP 800 mV. When the contact time increased to 60 min, the reduction of *E. coli* count was about $3 \log_{10}$ CFU / ml in ORP 800 mV, $4 \log_{10}$ CFU / ml in ORP 950 mV. and $6 \log_{10}$ CFU / ml in ORP 1100 mV. These results could be concluded that the ORP of EO water and contact time significantly inactivated *E. coli*.

Key words: electrolyzed – oxidizing water, *Escherichia coli*, holy basil

INTRODUCTION

In recent years, there has been increasing concern on food safety especially products that are consumed fresh or slightly cooked. In Thailand, these is the problem on contamination of *Escherichia coli* and *Salmonella* spp. in exported vegetables (peppermint, sweet basil, holy basil and coriander). Norway has rejected the exported vegetables from Thailand. DG SANGO asked for Thai competent authorities to do action plan for

control this microbial contamination. *E. coli* is food borne pathogen of major public health concern in many countries. A variety of foods, including poultry, egg, meat, milk, fruit and vegetables have been implicated as vehicle of one or more of these pathogens in outbreaks of food borne illness. (Venkitanarayanan *et al.*, 1999)

Electrolyzed oxidizing water is a relatively new method developed in Japan, and has been utilized in agriculture, livestock management,

medical sterilization, food sanitation and areas that rely on antimicrobial methodologies, (Venkitanarayanan *et al.*, 1999 ; Kim *et al.*, 2000b) Electrolyzed oxidizing (EO) water is created by electrochemical dissociation of salt water. This process splits salt water into two separate streams, acidic and alkaline water. Electrolytic acidic water provides more than 80 times the antimicrobial efficacy than sodium hypochlorite solutions due to its very high oxidation – reduction potential (ORP). When ORP is greater than 650 millivolts, most pathogens are killed . The ORP of the acidic water is greater than 1100 millivolts.

The disinfection capacity of EO water is due to its low pH and high ORP, and the relative concentrations of aqueous molecular chlorine, chlorine hypochlorous acid (HOCl), and hypochlorite ion (OCl⁻) (Ratna R. *et al*, 2003). These factors are related to the current input to the EO water generator. Since the use of EO water as a disinfectant is still being investigated, not much information is available about its antimicrobial activity. Unlike antimicrobial chemical, EO water has the advantage of having less adverse effects to environment, because it is produced from pure water and NaCl (99% pure table salt). (Kim *et al.*, 2000b)

EO water is relatively new disinfecting compound that shows promise against all suspensions of *Escherichia coli*, *Salmonella enteritidis* and *Listeria monocytogens* attached to the cutting boards. (Venkitanarayanan *et al.*, 1999), spoilage organisms associated with vegetables (Izumi, 1999), pathogens in solution (Fabrizio and Cutter, 2004) or pathogens attached to poultry surface (Hoon *et al*, 2002)

Recently, Mueller *et al.* (2003) showed that EO water can be used to provide control of a fungal disease in the green house. Izumi (1999) showed that EO water was used to as disinfectant for fresh cut vegetable. Ratna and Ali (2003) showed that EO water could be used to reduce *E. coli* O157 : H7 on the sprouts about 88.9% (0.96

log₁₀ CFU/g) with a control treatment 32 min.

This research was undertaken for the potential use of electrolyzed oxidizing water to treat holy basil inoculated with *E. coli*. The main objective was to improve the antimicrobial treatments of EO water to holy basil inoculated with *Escherichia coli* by optimizing the procedure. Treatments were conducted for different contact times and different ORP.

MATERIALS AND METHODS

1. Preparation of *E. coli*

The strain of *E. coli* was obtained from the culture collection of Institute of Food and Research and Product Development. The bacterial cells were grown in 100 ml of tryptic soy broth (Merck, Darmstadt Germany) and incubated at 37°C for 24 h, and diluted in 1 ml of sterile 0.1% peptone water.

2. Inoculation of *E. coli* pure culture and treatments

A volume of 1 ml of each bacterial culture was transferred to 9 ml of EO water (treatment) or sterile deionized water (control) in screw – cap tubes. The tubes were incubated at room temperature (25°C) for 10, 30 and 60 min. After that the number of viable cells in each sample was determined by direct plating method, using 0.1 ml portions directly or after serial (1 : 10) dilutions in 0.1% peptone water on tryptic soy agar plates and incubated at 37°C for 24 hr.

3. Inoculation of *E. coli* in holy basil

Holy basil were obtained from a local grocery market. Two hundreds and fifty grams of holy basil were soaked in the suspension of *E. coli* (7.7 log 10 CFU/ml) for 10 min with gentle agitation. After inoculum was decanted, the holy basil was placed on sterile perforated trays and dried in a laminar flow hood at room temperature for 1 hr. The population of *E. coli* per gram of

holy basil was approximately $8 \log_{10}$ CFU.

4. Preparation of electrolyzed oxidizing water

EO water was produced with EO water generator (Zimmermann Verfahrenstechnik AG, Switzerland). A continuous supply of EO water and 16% sodium chloride solution at room temperature was pumped into the equipment operating at 5 to 6 A. The pH and oxidation – reduction potential (ORP) for the EO water were determined by a pH/ORP meter (Model HI 98121). The EO water were diluted with sterile water to be ORP 800, 950 and 1100 mV.

5. Treatment of holy basil with EO water

Five grams of holy basil inoculated with *E. coli* were treated with 100 ml of EO water using three ORP levels of EO water, i.e., 800, 950 and 1100 mV. The treatments were conducted for 10, 30 and 60 min to determine the effect of treatment time. All experiments were carried out in replicates for three time.

6. Microbiological analysis

To determine the total count of *E. coli*, ten grams of untreated and treated holy basil, were placed in 90 ml of sterile 0.1% peptone water in a stomacher for 2 min. After that, the wash solution was serially diluted in sterile distilled water. Each dilution was pipetted to a sterilized plate (1 ml) and poured plate with tryptic soy agar. After incubating at 37°C for 24 h, presumptive *E. coli* colonies were enumerated.

RESULT AND DISCUSSION

The pH and ORP of EO water are presented in Table 1. The initial ORP and pH of EO water were 2.09 and 1200 mV. When EO water was diluted to 1 : 1, 1 : 5 and 1 : 50, the final ORP and pHs were 1100, 2.6; 950, 4.10 and 800, 7.28, respectively. The ORP level increased with decreasing pH. The reason might be explained as

acidic pH (pH < 5), where HOCl constitutes more than 97% of the total chlorine (White, 1999), the ORP level increased with decreasing pH due to the Nernst Law (Oldham and Mayland, 1994). When the pH is above 5.0, the fraction of OCl⁻ had less oxidative and microbial effects than HOCl. There are studies showing that the ORP level of EO water is more essential than low pH to reduce the survival of microorganisms. (Kim *et al.*, 2000b; Hoon *et al.*, 2004)

The initial viable cell of *E. coli* pure culture used for this study was approximately $8.5 \log_{10}$ CFU / ml. When this culture was treated with ORP levels of EO water 800, 950 and 1100 for 10, 30 and 60 min, the viable cells were reduced to undetectable levels at every ORP levels and length of times used mV.

The results of survival of *E. Coli* in inoculated holy basil treated with various ORP levels EO water and time are shown in Table 3.

The initial population of *E. coli* used for inoculated on holy basil was approximately $8.5 \log_{10}$ CFU / ml. When the inoculated holy basil were treated with EO water, all the treatments were significantly lower than the untreated sample. However no reduction in bacterial counts was achieved in the control sample (treated with deionized water). The EO water containing ORP level of 800 mV could reduce bacterial counts for $2 \log_{10}$ CFU / g when the holy basil was treated for 10 and 30 min. The similar ORP level could reduce bacterial count for $3 \log_{10}$ CFU / g when treated for 60 min. When the EO water containing

Table 1 Effect of dilution on pH and ORP levels of EO water.

Dilution of EO water :		
Water	pH	ORP (mV)
1 : 0	2.09 ± 0.02	1200
1 : 1	2.60 ± 0.05	1100
1 : 5	4.10 ± 0.07	950
1 : 50	7.28 ± 0.04	800

Table 2 Surviving population of *E. coli* treated with various ORP levels of EO water for 10, 30, 60 min^a.

Contract Time (min)	Surviving population (log ₁₀ CFU / ml)			
	Control ^b	800 mV	950 mV	1100 mV
10	7.72 ± 0.03	ND ^c	ND	ND
30	7.67 ± 0.05	ND	ND	ND
60	7.59 ± 0.12	ND	ND	ND

^a Values are the means of three replicated ± SD

^b Deionized water

^c No detectable survivors by direct plating method

Table 3 Surviving population of *E. coli* treated on holy basil when were treated with various ORP levels of EO water for 10, 30 and 60 min^a.

Contract Time (min)	Surviving population (log ₁₀ CFU / g)			
	Control ^b	800 mV	950 mV	1100 mV
10	8.45 ± 0.04	6.29 ± 0.11	6.27 ± 0.08	4.72 ± 0.01
30	8.33 ± 0.12	6.81 ± 0.05	5.16 ± 0.02	3.25 ± 0.03
60	8.29 ± 0.08	5.59 ± 0.03	4.79 ± 0.03	2.56 ± 0.13

^a Values are the means of three replicated treatments ± SD

^b Deionized water

ORP 950 mV and 1100 mV was used for 30 min, bacterial counts were reduced for 3 log₁₀ CFU / g and 5 log₁₀ CFU / g, respectively. The most reduction was the treatment with ORP 1100 mV EO water and incubated 60 min, which reduced *E. coli* for 5 log₁₀ CFU / g. This results showed that the higher ORP levels of EO water and longer time of treatments appeared to be responsible for greater antimicrobial effect than lower ORP and shorter time.

The mechanisms of microbial cell inactivation was not clear, but it was believed to involve the presence of hypochlorous acid and the high ORP levels of EO water. Previous studies suggested that hypochlorous acid inactivated microorganisms by (1) inactivation of enzymes involved in respiration (Albrich *et al.*, 1986 ; Hurst *et al.*, 1991), (2) oxidation of cell surface sulfhydryl compounds (Leyer and Johnson, 1997), (3) inhibition of ATP generation (Barrette *et al.*, 1989), (4) retardation of active transport cell (Albrich *et*

al., 1986; Hurst *et al.*, 1991). However, the achievement of EO as antibacterial agent for pure culture of *E. coli* was greater than in the inoculated *E. coli* on holy basil. This could be explained by report of Hoon *et al.*, (2002) that the pathogens were attached to skin interface, entrapped in folds, crevices and pores of animals and vegetables, thus the disinfectants could not penetrate into the crevices or pores that appeared to protect cells.

This study concluded that ORP levels of EO water and contact time affected the survival of *E. coli* population in both pure culture and inoculated holy basil. The EO water can inactivate *E. coli* both pure culture and inoculated holy basil.

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