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Research Article

Electrolyzed water as an antibacterial agent for washing fresh chicken meat

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Abstract

The effectiveness of electrolyzed water (EO) as an antibacterial (*Escherichia coli* and *Salmonella typhi*) agent for washing fresh chicken meat was investigated. EO water (5% NaCl, 8 A., 15 min) containing 30 ppm of residual chlorine was able to effectively inhibit growth of *Escherichia coli* and *Salmonella typhi*. EO water was further investigated as a possible antibacterial agent for washing fresh chicken meat. The population of *Escherichia coli* and *Salmonella typhi* in fresh chicken meat was reduced to less than 2 $\log_{10}$ CFU/g after washing with EO water. Shelf life study of chicken wings inoculated with *E. coli* and *S. typhi* treated with EO water were reduced by nearly 2 $\log_{10}$ CFU/g for up to 2 days. Sensory evaluation test using hedonic scale reveled that fresh chicken meat washed with EO water possessed hedonic scale of 7.0±1.8 (like moderately). This finding suggests that EO water has good potential as an antibacterial agent for washing fresh chicken meat in the food industry.

Keywords: spoilage, contamination, *Escherichia coli*, *Salmonella typhi*, Thailand.

Introduction

Escherichia coli and *Salmonella typhi* are pathogenic bacteria commonly found on seafood [1], pork [2], and also fresh chicken. *Escherichia coli* and *Salmonella typhi* have been responsible for significant illness from consumption of affected meat [3]. Illness is usually acquired by ingestion of contaminated water or poultry products. Development of strategies to control *Escherichia coli* and *Salmonella typhi* has been studied for a number of years. Electrolyzed oxidizing water (EO) has been regarded as a novel antimicrobial agent in more recent time. It is usually generated by electrolysis of a dilute NaCl solution in a chamber with anode and cathode electrodes separated by a membrane and obtained from the anode side [4]. EO water has been proven to exhibit strong bactericidal activity to many pathogens [5, 6]. The objective of this work was designed to evaluate the effectiveness of EO water for reducing *Escherichia coli* and *Salmonella typhi* on fresh chicken during washing. This also involved the sensory evaluation of the boiled chicken.

Materials and Methods

Bacterial culture preparation

Escherichia coli (WU 20081) and *Salmonella typhi* (WU 20085) were obtained from Food Microbiology Laboratory, Walailak University (Nakhon Sri Thammarat, Thailand) and were identified from poultry products. Each strain was grown on nutrient agar (Merck Ltd, Thailand) at 35°C for 24 h. The bacterial population in all the inoculated media was standardized to 10^8 CFU/ml after 48 h incubation.

Antimicrobial activity testing in pure culture

Generation of EO water involved electrolysis of sodium chloride in a cell containing inert positively charged and negatively charged platinum electrodes separated by a bipolar membrane. A salt solution (1% and 5% NaCl) and deionized water (control) were pumped into the EO water generator by subjecting the electrodes to direct current (6, 8, 10 A) for 5, 10, 15, 20, 25 and 30 min. The effect of treatment time on bactericidal activity was performed by adding 1 ml of *Escherichia coli* and *Salmonella typhi* (approximately 10^8 CFU/ml) into the sterile screw-cap tubes which contained 9 ml of each EO water or sterile deionized water (control). The tubes was shaken using a platform shaker at 200 rpm. After 5 min, the viable count of *Escherichia coli* and *Salmonella typhi* in each sample was determined by plating 0.1 ml portions directly or after serially diluted in sterile 0.1% peptone water on Compact Dry "Nissui" EC (for *E. coli*), and Compact Dry "Nissui" SL (for Salmonella). All of Compact Drys were purchased from Oskon Ltd, Thailand. *E. coli* and *Salmonella typhi* were incubated at 35°C for 24 h before counting.

Antimicrobial activity testing on fresh chicken

Middle sections of chicken wings (approximately 45±5 g per sample) were purchased from a local market, Thasala District, Nakhon Sri Thammarat and stored at 4°C for no more than 1 h before testing. Samples were removed aseptically from packaging immediately prior to treatment by sanitizing the packaging surface with 70% ethanol and a sterile scalpel. Chicken was surface treated with UV light in a biological safety hood on sterile racks. Surfaces were exposed evenly by turning every 10 min for up to 30 min. Five ml of the *E. coli* and the *S. typhi*, containing approximately 8 $\log_{10}$ CFU/ml, was inoculated onto UV-treated chicken surfaces by spray inoculation with a hand-held spray bottle under a biological safety hood. The bacterial culture was allowed to attach to chicken wing surfaces for 15 min prior to any treatments. Using this procedure, approximately 8 $\log_{10}$ CFU/g of pathogen was obtained on chicken surfaces. After inoculation, chicken were dipped in 500 ml of EO (5% NaCl, 8 A., 15 min). Following treatments, 25g samples of inoculated chicken were placed in an incubator and shaken gently (100 rpm) at a room temperature of (30±2°C) for 10 min. At the end of the treatment, the viable cells in washed treatment solutions and neutralizing buffer solution were assayed through serially diluting in 9 ml of sterile 0.1% peptone water and then directly plating 0.1 ml of each dilution in duplicate on Compact Dry "Nissui" EC (for *E. coli*), Compact Dry "Nissui" SL (for *Salmonella*).

Experimentally inoculated chicken was dipped in 500 ml of EO water for 15 min at 30°C and allowed to drip for 60 s. Following treatments, chicken was individually vacuum-packaged, stored at 4°C and sampled at days 0, 1, 4 and 7. Sampling and microbiological analyses were performed as described above.

Sensory evaluation

Fresh chicken wings were dipped in an aqueous solution of EO water (5% NaCl, 8 A., 15 min) containing 30 ppm of residual chlorine. The chicken was kept submerged for 20 min and then air dried at room temperature for 5 min. Chicken meat was boiled by hot water at 98 ± 2°C for 360 s. After boiling, samples were allowed to drain for a short time.

The samples were subjected to sensory analysis by an untrained panel (56 panelists for each test) using hedonic scale. Panelists were selected from students and staff at Walailak University, Nakon Sri Thammarat. A 9-point hedonic scale ranging from "like extremely" to "dislike extremely" was used to determine their degree of acceptance of chicken in terms of colour, flavour, taste, texture and overall liking. Sensory results were expressed as mean. All variables were tested for normality and homogeneity of variance to meet the assumptions required for ANOVA. The data were statistically analysed by one-way ANOVA and Duncan's post hoc test; $P < 0.05$ was considered to be statistically significant.

Results and Discussion

Antimicrobial activity testing in pure culture

The reduction in *E. coli* and *S. typhi* population as a result of treatment with EO at different concentration of sodium chloride and electric current exposure time are shown in Table 1. For each treatment the difference in EO water potency against *E. coli* and *S. typhi* can be observed.

Table 1. Inactivation of *Escherichia coli* and *Salmonella typhi* cultures by EO water.

Salt content (%)	Current (A)	Surviving population of bacteria a in period of time (min)											
		Escherichia coli (CFU/mL)[a]						*Salmonella typhi* (CFU/mL)[a]					
		5	10	15	20	25	30	5	10	15	20	25	30
1	6	-	-	10^4	10^2	<10	<1	-	-	10^4	10^2	<10	<1
	8	-	-	10^2	<1	<1	<1	-	-	10^4	<1	<1	<1
	10	-	<1	<1	<1	<1	<1	<10	<1	<1	<1	<1	<1
5	6	-	10^2	<1	<1	<1	<1	-	-	<10	<1	<1	<1
	8	-	<1	<1	<1	<1	<1	-	<1	<1	<1	<1	<1
	10	-	<1	<1	<1	<1	<1	-	<10	<1	<1	<1	<1
Control		10^6	10^6	10^6	10^6	10^6	10^6	10^6	10^6	10^6	10^6	10^6	10^6

[a]The initial bacteria populations is approximately 10^8 cfu/ml

-: not examined

<1 cfu/ml: Not detected in 1 mL of sample

<10 cfu/ml: Not detected in 1 mL of the 1 in 10 dilution of sample

EO water reduced the populations of *E. coli* and *S. typhi* to lower levels with respect to control (untreated sample). EO achieved a higher reduction of both *E. coli* and *S. typhi* at 5% NaCl than that achieved at 1%NaCl. An exposure time of 15 min, 8A achieved the lowest value of 0 (ND, not detected). Increasing exposure time enhances the effects of the anti-microbial activity of EO due to longer action time on the bacterial cell by inactivating it. However, long treatment times adversely affect the physical appearance and nutritional content of the products. The free chlorine concentration of each treatment (EO) used at each treatment time are shown in Table 2. EO water produced at 5 %NaCl, 8 A, 15 min had available chlorine concentration of 30 ppm.

Antimicrobial activity testing on fresh chicken

Survival characteristics of *E. coli and S. typhi* on chicken wings treated with EO water (5% NaCl, 8 A., 15 min) and deionized water (control) are shown in Figure 1. *Escherichia coli* (<2 $\log_{10}$CFU/g) and *Salmonella typhi* (<2 $\log_{10}$CFU/g) were found on the EO treated chicken samples. The initial population of *E. coli* and *S. typhi* inoculated on each sample was about 8 $\log_{10}$ CFU/g. For EO treatment, the populations of *E. coli* and *S. typhi* were reduced by 4-5 $\log_{10}$ CFU/g, whereas the control resulted in 2-3 $\log_{10}$ CFU/g.

Table 2. Residue chlorine concentration of electrolyzed water using different treatment time.

% of salt content	Electric current (A)	Residue of chlorine (ppm.) Time (min)					
		5	10	15	20	25	30
1	6	-	-	10	20	40	50
	8	-	-	20	50	50	60
	10	-	20	50	50	60	60
5	6	-	-	10	20	60	60
	8	-	-	30	40	40	60
	10	-	-	20	40	50	60

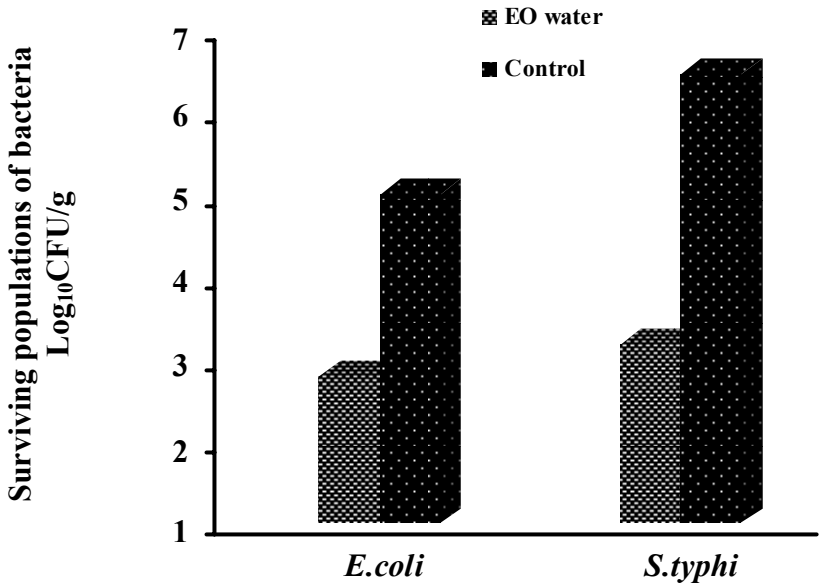
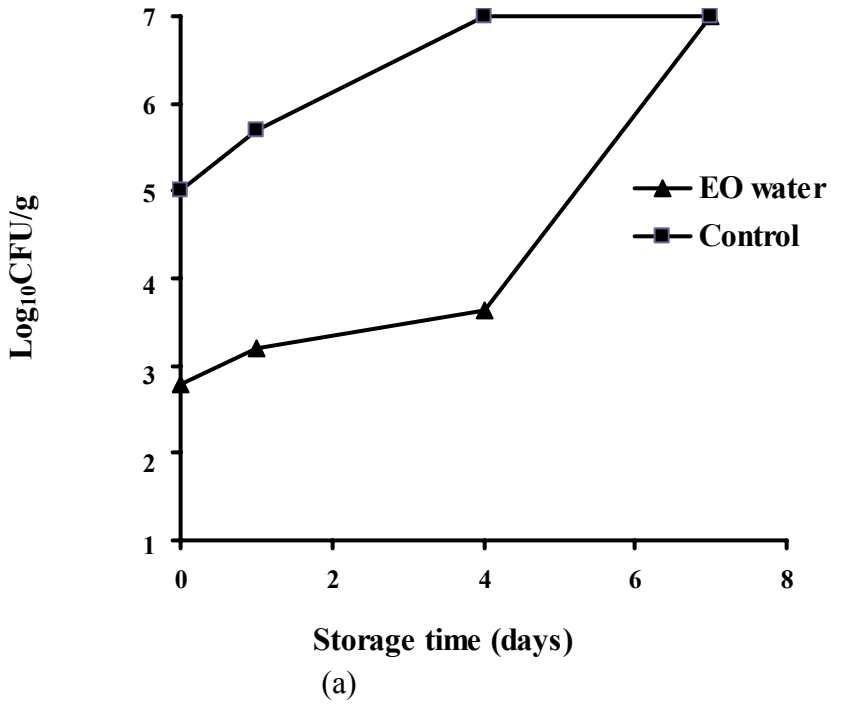
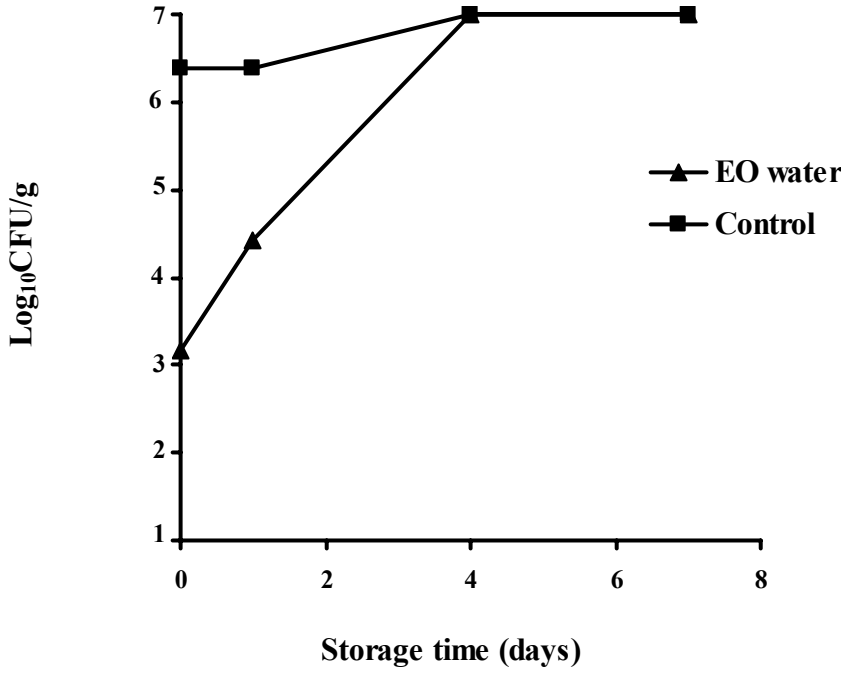


Figure1. Surviving populations of *E. coli* and *S. typhi* after washing with EO water.

Immediately after dipping with EO for 15 min at 5% NaCl and 8 A., *E. coli* and *S. typhi* were reduced by nearly 4-5 $\log_{10}$ CFU/g (Figure 2). This reduction was maintained for up to 1-2 days. However, by day 4, the reduction diminished as bacterial populations increased. However, EO-treated chicken had lower populations of *E. coli* and *S. typhi* as compared to untreated samples on days 1 to 3. Application of EO as an inhibitor of microorganisms in food products has continually attracted much attention in a recent years in response to consumer concerns about the use of artificial chemical preservatives [7]. These results showed that the EO treatment had good potential for use in protecting poultry meat from bacteria and may possibly be applied in the food industry.



(a)



(b)

Figure 2. Shelf life study of chicken wings inoculated with *E. coli* (a) and *S. typhi* (b) and treated with EO water for 15 min at 5% NaCl, 8 A and stored at 4°C.

Sensory evaluation

The panelists evaluated treated boiled chicken including the control. The responses were converted to numerical values ranging from 1 for "dislike extremely" to 9 for "like extremely". Sensory evaluations of control boiled chicken and treated boiled chicken are summarised in Figure 3. The panel could not distinguish overall liking scale between control chicken and those preserved with

EO (*P>0.05*). In addition, no significant differences of flavour, colour, taste and texture (p<0.05) in consumer hedonic test were found between boiled chicken with EO and without EO.

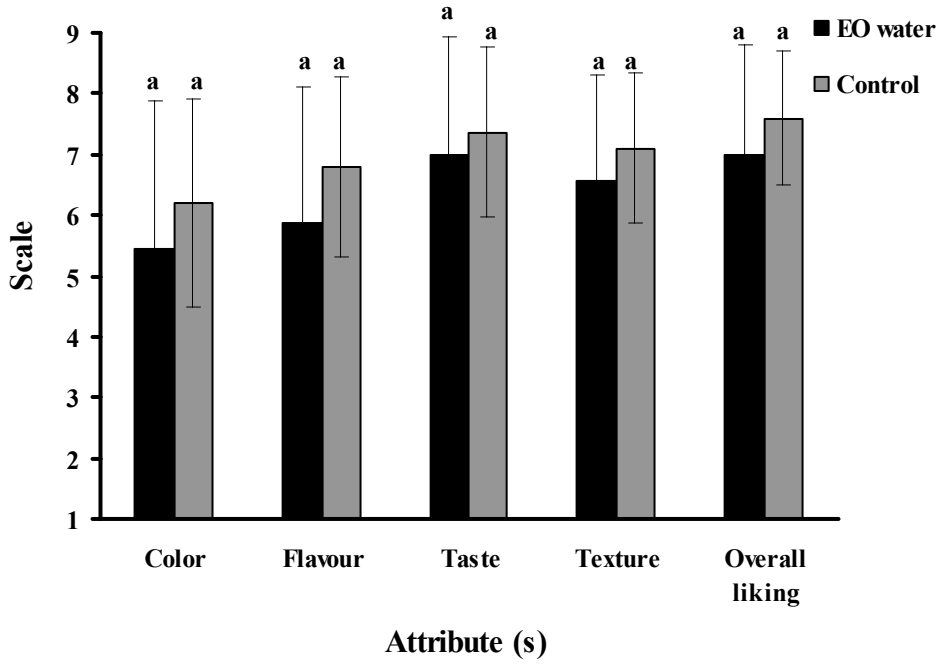


Figure 3. Means of hedonic scores of attributes for fresh chicken meat washed with EO water (5% salt, 8 A, 15 min).

[a] Mean value by the same letter are not significantly (P<0.05) different according to Duncan test following ANOVA

Conclusion

This study demonstrates that EO water was capable of reducing populations of *Escherichia coli* and *Salmonella typhi* >6 $\log_{10}$ CFU/g on experimentally inoculated chicken wings. The information obtained from this study may be useful to researchers who are interested in identifying additional inexpensive and/or other practical methods to enhance the microbiological safety of poultry products.

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