
Effectiveness of different sanitizers in inactivating *E. coli* O157:H7 in Tomato and Cucumber

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Abstract: Fresh produce is popular worldwide because it is recognized as an important source of nutrients, vitamins and fiber for humans. However, in the last two decades outbreaks of food borne illness and cases associated with fresh produce have increased significantly. This study was conducted to evaluate the efficacy of acidic electrolyzed water (EO) and alkaline water (AlcO), 200-ppm chlorine water, 3% H₂O₂ and/or sterile distilled water wash in reducing *Escherichia coli* O157:H7 on the surface of dip-inoculated tomato and cucumber. Inoculated sample (tomato, cucumber) were dipped in 200 ml of electrolyzed acidic or alkali water, or 200 ppm chlorine water, or 3% H₂O₂ and/or sterile distilled water and hand rubbed for 90 seconds followed by second wash with sterile distilled water for 90 seconds to minimize the residual effect. Approximately 2.0 log CFU/g reduction was observed when washed with acidic electrolyzed water (EO) and alkaline water (AlcO). On the other hand, approximately 3.0-4.0 log CFU/g reduction was observed while washing with chlorine water and 3% H₂O₂. Washing with distilled water found ineffective in reducing the pathogen. Prevalence of total microbial population, coliforms, *Salmonella enteritidis*, *E. coli* O157:H7, *Listeria monocytogenes*, *Yersinia enterocolitica* was also observed in non-spiked samples (tomato, cucumber). It was found that total microbial population, coliform bacteria and other pathogens varied among different samples depending on the location and type of samples. However, no *Salmonella* spp., were found in the tested samples. The results revealed that 3% H₂O₂ or 200 ppm chlorine water gave better reduction than that of acidic electrolyzed water and alkali water.

Keywords: Fresh Produce, Sanitizers, *E. coli* O157:H7, and Electrolyzed Water

1. Introduction

Fresh fruits and vegetables are an essential part of the diets of people around the world. Nutritionists emphasize the importance of fruits and vegetables in a healthy diet, and researchers have recommended the consumption of at least five servings per day. Possibly as a result of these efforts, over the past decade there has been an increase in the consumption of fresh fruits and vegetables, concurrent with increased global distribution, which has made more varieties of produce available year-round. The US Food and Drug Administration (FDA) conducted several studies to determine the percentages of imported fresh produce contaminated with three pathogens: *Escherichia coli* O157:H7, *Salmonella* and *Shigella* spp (FDA, 2011). The FDA is currently conducting considerable similar research with domestic produce. Several outbreaks of human

diseases have been associated with the consumption of fresh fruits and vegetables contaminated with foodborne pathogens, such as *E. coli*, (1) *Listeria monocytogenes* (2) and *Salmonella* (3). Outbreaks of *E. coli*, *L. monocytogenes* and *Salmonella* and *Bacillus cereus* have been associated to the contaminated raw salad vegetables consumption, raw tomatoes (4) respectively. In developing countries like Bangladesh, agricultural practice of vegetable is quite unhygienic. Here vegetable are produced in open field or in homestead gardens. Sorting of the harvested vegetables in open ground and also washing those vegetables in roadside water source is a common practice in rural area of Bangladesh which is a potential source of microbial contamination. Transportation is another potential reason of contamination. Cross contamination causes from the

transportation of different types of vegetable from harvested site to local bazaar and to city market altogether in a single vehicle (5). And also vegetables are staked together for sell in vegetable market.

The sanitization of fresh fruits and vegetables plays an important role in the improvement of food quality and microbial safety, because the control of spoilage and pathogenic micro-organisms is critical throughout the production chain of growing, processing, distribution and consumption (6). Several researchers have tested numerous sanitizers for efficacy in reducing or killing pathogenic bacteria, such as *E. coli* O157:H7, *Salmonella* spp. and *L. monocytogenes*, on fresh fruits and vegetables (7). Chlorinated water is the most frequently and widely used disinfectant method for the washing of produce (8). Washing with chlorinated water, however, has a minimal sanitizing effect and results in a microbial reduction of <2.0 log CFU/g on fresh fruits and vegetables (9). A variety of other chemicals, including chlorine dioxide (ClO_2), hydrogen peroxide (H_2O_2), organic acids, trisodium phosphate, ozone and calcinated calcium solution, have been evaluated for their action against foodborne pathogens on fresh produce (10). Most of these sanitizers, however, are made from the dilution of concentrated solutions or powders, the handling of which involves some risk. More natural and less toxic alternatives are preferable. Moreover, they must be both simple and economical to use. Hence, there is a need for more efficacious and less hazardous sanitizers for suppressing pathogenic microorganisms on fresh fruits and vegetables (11).

Electrolyzed water (EW) is originated from electrolysis of a dilute salt solution (0.1% NaCl) passing through an anode and cathode electrodes containing electrolyzing chamber. Acidic electrolyzed water (AC-EW) containing hypochlorous acid and having a low pH (approx. 2.7) is produced at the anode. Alkaline electrolyzed water (AK-EW) which has a high pH (approx. 11.6), is produced at the cathode (12). Acidic EW, as a sanitizer has been found to produce significant reductions of various food borne pathogenic micro-organisms (13). It has been successfully used as a sanitizing agent not only in food produce but also in agricultural industries (14). The effectiveness of AC-EW in reducing pathogenic microorganisms on fresh vegetables has been examined by several researchers. It has been found successful when applied to reduce populations of *Salmonella typhimurium*, *E. coli* O157:H7, aerobic bacteria, *coliforms* and *B. cereus* on lettuce (15), *L. monocytogenes* on lettuce (16);

In our study, we evaluate the effectiveness of different sanitizer including 200 ppm Cl water, EW, H_2O_2 , and AK-EW that are readily available, in reducing food-borne pathogen in fresh produce. In addition, *E. coli* O157:H7 inoculated study was also done to compare the effectiveness in reducing the bacterial pathogen on the surfaces of cucumber, spinach and tomatoes.

2. Materials and Methods

2.1. Evaluation of Natural Microbial Content in Sample

2.1.1. Sample Collection

Commercial tomatoes and cucumber, were purchased from Shantinagar market, and used on the day of collection. Each tomato weighed 80.0 ± 0.5 g, cucumber weighed 110.0 ± 0.5 g and Spinach weighed 30g. All samples measured by an electric balance (Shimadzu Corporation, Japan). Injured or dirty samples were discarded.

2.1.2. Medium Used

Tryptic Soy Agar (TSA) medium was used to see the total bacterial count and other selective medium such as Sorbitol MacConkey (SMAC) agar for *Escherichia coli*, Chromogenic agar for *coliform*, Bismuth Sulfite Agar (BSA) for *Salmonella*, Cefexime Tellurite-Sorbitol MacConkey (CT-SMAC) for *E. coli* O157:H7, *Listeria* selective agar for *Listeria monocytogenes* and *Yersinia* selective agar for *Yersinia* spp., are also used.

2.1.3. Preparation of the Washing Solutions

All chemicals used for the preparation of the washing solution were purchased from Wako Pure Chemical Co. Ltd. (Osaka, Japan). Washing solutions were prepared immediately prior to application and used within 30 min after preparation. The chlorine solution was prepared using sodium hypochlorite (Wako Chemical, Japan) solution to distilled water (vol/vol), and concentration was adjusted to 200ppm. Distilled water (DW) was also used for experiments. Electrolyzed Acidic & alkaline water was generated with a model ROX-20TA EO generator & (0.1%) sodium chloride was used. The pH of acidic water was normally ≤ 2.7 & the pH of alkaline water was normally ≤ 11.25 . 3% H_2O_2 solution was prepared by using 30% H_2O_2 .

2.1.4. Washing Protocol.

In each experimental condition, 80 g of tomato, and 110 g of cucumber was washed separately with 200mL of the washing solution in a sterile Ziploc bag. Washing was carried out for 90s at room temperature by hand rubbing. After washing, the solution was decanted; a second wash was done with 200mL of sterile distilled water to remove the residue of the washing solution. After washing experiment, tomato, and cucumber were placed on a sterile perforated tray in a laminar flow biosafety cabinet for 4h at $22 \pm 2^\circ\text{C}$ to facilitate drying.

2.2. Procedure for Inoculation Study

2.2.1. Bacterial Test Strains

Strains of *E. coli* O157:H7 (CARS-3 isolated from bovine feces), were used in this study. To minimize the growth of microorganisms naturally present on vegetable samples, all the test strains were adapted to grow in tryptic soy broth (pH 7.3; Oxoid) supplemented with rifampicin (50 $\mu\text{g}/\text{mL}$). Plating on media containing rifampicin greatly minimized interference with colony development by naturally occurring

microorganisms and facilitate the detection of test pathogen on recovery media. Introduction of drug resistant mutations into test strains previously has been used effectively to detect inoculated bacteria in food products (Inatsu *et al.*, 2003).

2.2.2. Preparation of Inocula

E. coli O157:H7 was cultured at 37°C in 5 ml of tryptic soy broth (TSB; oxoid) medium supplemented with 50µg/ml rifampicin (TSB-Rif). Cultures were transferred to TSB-Rif by loop at three successive 24-h intervals immediately before they were used as inocula. Cells were collected by centrifugation (3000g, 10 min, 22°C) and resuspended in 9mL of sterile 0.85% sodium chloride. The inoculum (9 ml) with an initial concentration of approximately 10⁷CFU/ml was maintained at 22 °C ± 1°C and applied to the tomatoes within 1 hour of preparation.

2.2.3. Inoculation of Vegetable (Tomato, Cucumber)

Dip inoculation method was used to inoculate tomato, and cucumber with *E. coli* O157:H7. Six (6) pieces of each sample (Tomato, Cucumber) were dipped into 2L of *E. coli* O157:H7 inoculum in a beaker and mixed gently with a sterile glass rod for 20 min. This process was done inside the laminar flow biosafety cabinet. After the inoculum was decanted, samples were placed on a sterile Ziploc bag for washing experiments and were washed as described above.

2.3. Microbiological Analysis

The numbers of viable cells of various microorganisms in tomato under various experimental conditions were evaluated. Tryptic soy agar supplemented with 50 µg/ml rifampicin was used as a nonselective medium for determination of number of viable cells for all the pathogens tested. Samples containing *E. coli* O157:H7 were surface plated onto Sorbitol MacConkey agar supplemented with cefixime (0.05 mg/liter)

(CT-SMAC) and potassium tellurite (2.5 mg/liter) (CT-selective supplement, Oxoid) and 50µg/ml rifampicin. All ingredients except CT-selective supplement and rifampicin were combined and sterilized by heating at 121°C for 15 min. Supplement and rifampicin were added to the molten agar before pouring the medium into petri plates.

3. Result and Discussion

3.1. Effect of Sanitizer in Resident Microbial Population of Vegetables

In this study, five different sanitizers and two vegetable samples were used to evaluate the effectiveness of sanitizers in reducing total viable count in tomato and cucumber samples obtained from different market of Dhaka city. The prevalence of resident microorganisms and pathogens and the effectiveness of sanitizer in reducing these bacteria were presented in the Table 1. The high prevalence of total viable bacteria (4.5 log CFU/g), coliform bacteria (2.3 log CFU/g), and 1.9 log CFU/g of *E. coli*, 3.8 log CFU/g of *Staphylococcus aureus*, 3.8 log CFU/g of *Listeria* spp. and 1.7 log CFU/g *Yersenia* spp was recorded in tomato samples. However, no *salmonella* and *vibrio* spp was detected in the tomato samples. Washing with 200ppm chlorine water, electrolyzed acidic and alkaline water or H₂O₂ exhibited approximately 2.0 log CFU/g of reduction of resident bacteria and on the other hand, complete reduction of coliform, *E. coli*, *Staphylococcus aureus*, *Listeria* spp, *Yersenia* spp found in tomato samples. *Salmonella* spp., were not found on the tomato surface before enrichment, but after enrichment its presence was detected. This finding suggested that the fresh tomato samples collected from local markets were heavily contaminated with resistant bacteria and is of special concern for human consumption.

Table 1. Prevalence of pathogens in tomato samples and effect of washing with different sanitizer on pathogenic bacteria.

Pathogen of interest	Control	DW	Cl	Electrolyzed acidic W	Electrolyzed alkaline W	3%	DW	Cl	Electrolyzed acidic W	Electrolyzed alkaline W	3%
		wash	wash	wash + 2nd DW wash	wash + 2nd DW wash	H ₂ O ₂	wash	wash	wash + 2nd DW wash	wash + 2nd DW wash	H ₂ O ₂
After washing with sanitizers (Log ₁₀ CFU/gm)						After Enrichment					
Total viable count	4.5	3.3	2.5	2.3	2.7	2.0	ND	ND	ND	ND	ND
Total coliform count	2.3	2	≤1.0	≤1.0	≤1.0	≤1.0	ND	ND	ND	ND	ND
<i>E. coli</i>	1.9	1.8	≤1.0	≤1.0	≤1.0	≤1.0	ND	ND	ND	ND	ND
<i>Staphylococcus aureus</i>	3.8	2.6	1.8	≤1.0	≤1.0	1.4	ND	ND	ND	ND	ND
<i>Salmonella</i> spp.	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	+	+	+	+	+
<i>Vibrio cholerae</i>	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	-	-	-	-	-
<i>Listeria</i> spp.	3.8	3.3	1.2	1.2	1.4	1.4	ND	ND	ND	ND	ND
<i>Yersenia</i> spp	1.7	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	ND	ND	ND	ND	ND

Legend: DW= Distilled water; Cl= Chlorine water; W=Water; Acidic W= pH 2.59; Alkaline W= pH 11.59; ≤1.0= Below Detection limit. ND= Not done as bacterial count was above detectable limit; +=Present; -= Absent.

The cucumber samples was found less contaminated compared to tomato samples. As shown in Table 2, total coliform bacteria, *E. coli*, *E. coli* O157 were found on the cucumber surface before any type of enrichment. After washing with sanitizers the pathogenic bacteria count was

almost zero except for distilled water washed samples. However, despite of washing condition, all the pathogenic microorganisms including *Staphylococcus aureus*, *Salmonella* spp, *Listeria* spp showed their presence after enrichment. This finding suggested that enrichment step is a

must to evaluate the presence of opportunistic pathogen in any food samples.

Table 2. Prevalence of different pathogens in cucumber samples and effect of washing with different sanitizer on pathogenic bacteria.

Pathogen of interest	Control	DW wash	Cl wash	Electrolyzed acidic W wash + 2nd DW wash	Electrolyzed alkaline W wash + 2nd DW wash	3% H ₂ O ₂	DW wash	Cl wash	Electrolyzed acidic W wash + 2nd DW wash	Electrolyzed alkaline W wash + 2nd DW wash	3% H ₂ O ₂
				After washing with sanitizers (Log ₁₀ CFU/gm)					After Enrichment		
Total viable count	5.3	4.4	2.6	3.3	3.5	3.4	ND	ND	ND	ND	ND
Total coliform count	4.5	3.6	2.4	2.8	2.7	2.6	ND	ND	ND	ND	ND
<i>E. coli</i>	3.6	3.3	1.6	2.3	1.6	2.1	ND	ND	ND	ND	ND
<i>E. coli</i> O157	3.0	2.6	≤1.0	≤1.0	≤1.0	≤1.0	ND	ND	ND	ND	ND
<i>Staphylococcus aureus</i>	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	+	+	+	+	+
<i>Salmonella</i> spp.	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	+	+	+	+	+
<i>Vibrio cholera</i>	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	+	+	+	+	+
<i>Listeria</i> spp.	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	+	+	+	+	+
<i>Yersenia enterocolitica</i>	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	ND	ND	ND	ND	ND

Legend: DW= Distilled water; Cl= Chlorine water; W=Water; Acidic W= pH 2.59; Alkaline W= pH 11.59; ≤1.0= Below Detection limit. ND= Not done as bacterial count was above detectable limit; +=Present; -=Absent.

As described in methods and materials dipping method had been applied to introduce pathogen (*Escherichia coli* O157:H7) inoculums on tomato surfaces and effectiveness of the sanitizers (Electrolyzed acidic water, Electrolyzed alkaline water, H₂O₂ and chlorinated water) in reduction of experimentally introduced pathogen inoculums were studied. Strain examined was adapted to grow in the presence of 50μg

of rifampicin per milliliter, one of several markers used to evaluate the survival of bacterial pathogens in food products with potentially large numbers of interfering background microbiota. Antibiotic-resistant markers have been widely used in studies to determine the fate of pathogens in non-sterile foods, including fresh produce, meats, and milk.

Table 3. Total *Escherichia coli* O157:H7 count in tomato samples after experimental inoculation and count after sanitizer's treatment.

Medium used	Control	DW wash	200ppm Cl wash	Electrolyzed acidic W wash + 2nd DW wash	Electrolyzed Alkaline W wash+ 2nd DW wash	3% H ₂ O ₂
Log ₁₀ CFU/gm						
TSAR	4.47	3.40	≤1.0	≤1.0	≤1.0	≤1.0
SMACR	4.70	3.40	≤1.0	≤1.0	≤1.0	≤1.0)
Enrichment						
TSAR	ND	+	+	+	+	+
SMACR	ND	+	+	+	+	+

Legend: DW= Distilled water; Cl= Chlorine water; W=Water; Acidic W= pH 2.59; Alkaline W= pH 11.59; ND= Not done as bacterial count was above detectable limit; TSAR-Tryptic Soya Agar with 50μg rifampicin; SMACR-Sorbitol MacConkey with 50μg rifampicin;+=Present; -= Absent; ≤1.0= Below Detection limit.

Table 4. Total *Escherichia coli* O157:H7 count in cucumber samples after experimental inoculation and count after sanitizer's treatment.

Medium used	Control	DW wash	200ppm Cl wash	Electrolyzed acidic W wash + 2nd DW wash	Electrolyzed alkaline W wash+ 2nd DW wash	3%H ₂ O ₂
Log ₁₀ CFU/gm						
TSAR	4.5	3.2	≤1.0	≤1.0	3.0	2.5
SMACR	4.0	2.2	≤1.0	≤1.0	2.5	≤1.0
Enrichment						
TSAR	ND	+	+	+	+	+
SMACR	ND	+	+	+	+	+

Legend: DW= Distilled water; Cl= Chlorine water; W=Water; Acidic W= pH 2.59; Alkaline W= pH 11.59; ND= Not done as bacterial count was above detectable limit; TSAR-Tryptic Soya Agar with 50μg rifampicin; SMACR-Sorbitol MacConkey with 50μg rifampicin;+=Present; -= Absent; ≤1.0= Below Detection limit.

About 4.5 log CFU/g of *E. coli* O157:H7 were recorded in inoculated tomato samples (Table 3). Dipping method was used as this inoculation method was better than any inoculation method used in spiked study. Washing with electrolyzed acidic water, electrolyzed alkaline water,

3%H₂O₂ and 200ppm chlorinated water was able to completely reduced the *E. coli* O157:H7 count compared to non-washed samples, whereas, distilled water wash could reduce only 1.0 log CFU/g compared to non-washed samples. Similar experimental findings were recorded for cucumber

samples as shown in Table 4. However, after enrichment study, the presence of *E. coli* O157:H7 was detected in all the samples. This finding suggested that sanitizer treatment could be able to reduce the bacteria on the surfaces of fresh produce, but were unable to eliminate the bacteria completely.

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