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Comparison of the Bactericidal Effect of Slightly Acidic Hypochlorous Water with That of Conventional Sterilizers

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The bactericidal effect of slightly acidic hypochlorous water (SAHW) on *Salmonella* Enteritidis, *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus cereus*, as well as some bacterial strains isolated from fresh lettuce was evaluated. Viable counts of all tested bacterial samples decreased immediately after treatment by SAHW. Most bacterial cells with the exception of *B. cereus*, and *S. aureus* were not culturable on TSA after treatment by 1 to 30 mg/L SAHW. Likewise, *Pseudomonas* sp., and *Flavobacterium* or *Xanthomonas* sp., *Kurthia* sp., *Micrococcus* sp., and *Corynebacterium* or *Microbacterium* sp. were not culturable on TSA after treatment by 30 mg/L SAHW. Viable counts of *S. aureus*, *E. coli*, *Flavobacterium* or *Xanthomonas* sp., and *Pseudomonas* sp. showed a 5 to 6 log cfu/mL reduction at day 0 and maintained a count of less than 1 log cfu/mL from day 1 to day 7 following treatment by 30 mg/L SAHW. Sodium hypochlorite (NaOCl, 0.5–1.0 mg/L) decreased the viable counts of *S.* Enteritidis to less than the lower limit of detection, 1 log cfu/mL, from day 1 to day 7 following treatment by 1 mg/L. NaOCl was not sufficient at 0.5–0.75 mg/L in reducing viable counts of *S.* Enteritidis because of a 2 to 5 log cfu/mL increase from day 2 to day 5 due to recovery from injury. Initial counts of *S.* Enteritidis after hydrogen peroxide (H_2O_2, 1000–2000 mg/L) treatment slowly decreased over time to less than 1 log cfu/mL after day 2. Treatment by 1750 to 2000 mg/L H_2O_2 has sufficient bactericidal activity on *S.* Enteritidis cells, however, at a higher concentration compared to NaOCl or SAHW. SAHW decreased viability of *S.* Enteritidis immediately with higher reduction counts in 1, 5, and 30 mg/L from day 0 to day 7 unlike NaOCl and H_2O_2.

INTRODUCTION

Increasing demand for fresh, healthy and convenient foods has led to a continuous growth in the fresh–cut fruit and vegetable industry (Rico *et al.*, 2007). Fruit and vegetable consumption is an important part of a healthy diet for consumers worldwide, but eating uncooked produce is not risk free (Rahman *et al.*, 2010). Fresh–cut produce is highly susceptible to microbial contamination from a number of sources including postharvest handling and processing. *Salmonella* Enteritidis, pathogenic *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus cereus* have been reported as common food–borne pathogens that are of major public health concern (Mead *et al.*, 1999). In Japan, 2007 statistics by the Japanese Ministry of Health, Labor and Welfare reported an estimated 1000 outbreaks of food–borne illness comprising about 4,000 cases associated with composite ready–to–eat foods and about 1000 cases with vegetable and vegetable products (Soli *et al.*, 2010).

Decontamination of fresh–cut produce plays an important role in the preservation of food quality and safety of consumption (Nguyen–The and Carlin, 1992). Unfortunately, the 'fresh' nature of produce restricts the use of thermal decontamination as a means of prevention, or elimination of risks associated with produce, or reduction of pathogenic bacteria to an acceptable level (Soli *et al.*, 2010). In Japan, sodium hypochlorite (NaOCl) solution (100–200 mg/L of available chlorine), acidic electrolyzed water (AcEW) (20–60 mg/L of available chlorine) and slightly acidic electrolyzed water (SlAEW) (10–30 mg/L of available chlorine) have been authorized for use with food by the Japanese Ministry of Health and Welfare (Koide *et al.*, 2009).

SlAEW has attracted growing interest concerning studies in washing and sanitizing treatments for the removal or inactivation of pathogens on food, as well as food contact surfaces (Suzuki *et al.*, 2005; Ono, Miyake, and Yamashita, 2005; Yamashita *et al.*, 2005; Okamoto., 2006; Koide *et al.*, 2009). Slightly acidic electrolyzed water or slightly acidic hypochlorous water (SAHW) (10–30 mg/mL of available chlorine) (Soli *et al.*, 2010) contains mainly hypochlorous acid (HOCl; 97%), which has been shown to have strong antimicrobial activity (Yoshifumi, 2003). The application of SAHW minimizes human health and safety issues from Cl_2 off–gassing, reduces corrosion of surfaces and limits phototoxic side effects while maximizing the application of HOCl species (Guentzel *et al.*, 2008), thus an alternative to using sodium hypochlorite (a widely used food product disinfectant) or hydrogen peroxide (H_2O_2).

The objectives of this study were to (1) investigate the bactericidal effect of SAHW against some Gram–negative and Gram–positive pathogens, such as *Salmonella* Enteritidis, *Escherichia coli*, *Staphylococcus*

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aureus, *Listeria monocytogenes*, and *Bacillus cereus* and against bacterial strains isolated from lettuce, a leafy vegetable being the prime ingredient in ready–made salads, and (2) comparing the bactericidal effect of SAHW with that of NaOCl and H_2O_2 solution.

MATERIALS AND METHODS

Bacterial strains

Salmonella Enteritidis IFO 3313, *Escherichia coli* IFO 3301, and *Staphylococcus aureus* IFO 3060 were purchased from Institute for Fermentation, Osaka, Japan. *Bacillus cereus* JCM 2152 was obtained from Japan Collection of Microorganisms, Wako, Saitama, Japan. *Bacillus cereus* emetic and diarrheal strains, *Escherichia coli* O157:H7, *Listeria monocytogenes* serotype 1/2a and 4b strains were provided by Fukuoka City Institute for Hygiene and the Environment, Fukuoka, Japan. *Flavobacterium* or *Xanthomonas* sp., *Pseudomonas* sp., *Kurthia* sp., *Micrococcus* sp., and *Corynebacterium* or *Microbacterium* sp. were isolated from lettuce and identified in our laboratory by using general biochemical tests (Gibbs and Skinner, 1966).

Preparation of SAHW

Fresh SAHW was generated using the Purester MP–240E Unit (Morinaga Milk Industry, Japan) at a production capacity of 240 L/h. The Purester unit electrolyzes about 2 to 6% of hydrochloric acid (HCl) in a non–diaphragm electrolytic cell generating a highly concentrated hypochlorous acid (HOCl), which is diluted by tap water, thus, producing SAHW of available chlorine concentration of 10 to 30 mg/L with pH 5.0 to 6.5. Further dilutions using deionized water were carried out to produce concentrations lower than 10 mg/L.

Bacterial culture and bactericidal test

Stock cultures of each pathogenic strain and bacterial isolates from lettuce were transferred into tryptic soy broth (TSB, Becton Dickinson) and incubated at 35 ºC for 24 h. Following incubation, cells were harvested from 10 mL of each culture by centrifugation at 5000×*g* for 5 min at 4 ºC and washed with sterile deionized water. The cells were then suspended in 10 mL solution of SAHW (0.1–30 mg/L of available chlorine concentration) at a final cell concentration of 10^7–10^8 cfu/mL. The cell suspensions were kept at 25 ºC for 5 min to determine minimal bactericidal concentration. For determination of the long–term effects of SAHW, the cells were suspended in 10 mL solution of SAHW (0.1–30 mg/L of available chlorine concentration) at a final cell concentration of 10^7–10^8 cfu/mL, and were kept at 25 ºC for 7 days. For comparison of the bactericidal effects of SAHW with those of NaOCl and H_2O_2 on *S*. Enteritidis, *S*. Enteritidis cells were suspended in 10 mL solution of SAHW (0.1–30 mg/L of available chlorine concentration), NaOCl (0.5–1.0 mg/L), or H_2O_2 (1000–2000 mg/L) at a final cell concentration of 10^7–10^8 cfu/mL. The cell suspensions were kept at 25 ºC for 5 or 7 days. Aliquots of the suspensions were withdrawn at suitable intervals, serially diluted 10–folds with sterile saline and 0.1 mL of diluted suspension were surface spread onto tryptic soy agar (TSA, Becton Dickinson). After incubation at 37 ºC for 48 h, viable counts were determined.

RESULTS

Bactericidal activity of SAHW on some pathogenic bacteria

Bactericidal activity of SAHW (1–30 mg/L of available chlorine) on pure cultures of some Gram–negative (*S*. Enteritidis, *E. coli*) and Gram–positive (*S. aureus*, *L. monocytogenes*, *B. cereus*) pathogenic and non–pathogenic bacteria is shown in Table 1. SAHW–treated cell suspensions (10^7–10^8 cfu/mL) of each bacterium were cultured and enumerated on TSA. Viable counts of all the pathogenic bacteria decreased immediately by 10^1–10^5 folds after treatment by 1.0 mg/L SAHW. As the chlorine concentration of SAHW was increased to 5, 10, and 30 mg/L, the viable counts further decreased. Most bacterial cells with the exception of *B. cereus*, and *S. aureus* were less than the lower limit of detection (<10 cfu/mL) by the plating method on TSA after treatment by 10 to 30 mg/L SAHW. Two strains of *B. cereus* (JCM 2152, diarrheal) had counts of 1.0×10^2 and 6.0×10^1 cfu/mL respectively after treatment by 30 mg/L SAHW. The bactericidal effect of treatment by 30 mg/L SAHW is sufficient in reducing the viable counts of *S*. Enteritidis, *E. coli*, *S. aureus*, *L. monocytogenes*, and *B. cereus*.

Table 1. Bactericidal activity of SAHW on some pathogenic and non–pathogenic bacteria

Bacteria	Viable counts (cfu/mL)				
	Control	After treatment with SAHW at (mg/L)			
		1	5	10	30
B. cereus JCM 2152	1.3×10^7	5.6×10^6	1.1×10^4	9.7×10^3	1.0×10^2
B. cereus emetic strain	1.7×10^7	1.0×10^4	5.5×10^3	1.7×10^3	<10
B. cereus diarrheal strain	1.4×10^7	1.4×10^4	1.5×10^3	1.2×10^3	6.0×10^1
L. monocytogenes serotype 1/2a	4.6×10^7	3.5×10^2	<10	<10	<10
L. monocytogenes serotype 4b	1.6×10^8	2.1×10^3	<10	<10	<10
S. aureus IFO 3060	7.0×10^7	3.1×10^5	6.6×10^4	9.4×10^2	<10
E. coli IFO 3301	4.1×10^7	2.2×10^3	6.0×10^1	<10	<10
E. coli O157:H7	3.6×10^7	1.0×10^3	<10	<10	<10
S. Enteritidis IFO 3313	1.4×10^8	1.1×10^6	<10	<10	<10

Table 2. Bactericidal activity of SAHW on some bacterial isolates from fresh lettuce

Bacteria	Viable counts (cfu/mL)				
	Control	After treatment with SAHW at (mg/L)			
		1	5	10	30
Flavobacterium or *Xanthomonas* sp.	2.1×10^8	5.3×10^6	1.1×10^4	9.7×10^3	<10
Pseudomonas sp.	1.1×10^8	1.5×10^5	5.5×10^3	<10	<10
Kurthia sp.	1.3×10^8	2.1×10^5	<10	<10	<10
Micrococcus sp.	1.2×10^8	<10	<10	<10	<10
Corynebacterium or *Microbacterium* sp.	1.1×10^8	<10	<10	<10	<10

Bactericidal activity of SAHW on some bacterial isolates from fresh lettuce

Bactericidal activity of SAHW (0.1–30 mg/L of available chlorine) on some bacterial isolates from fresh lettuce is shown in Table 2. SAHW–treated cell suspensions (10^7–10^8 cfu/mL) of each bacterium were cultured and enumerated on TSA. Viable counts of all the bacterial isolates from lettuce decreased by 10^2–10^3 folds after treatment by 1.0 mg/L SAHW. As the chlorine concentration of SAHW was increased to 5, 10, and 30 mg/L, the viable counts further decreased. *Flavobacterium* or *Xanthomonas* sp. had viable counts of 9.7×10^3 after treatment by 10 mg/L SAHW, however, *Pseudomonas* sp., *Kurthia* sp., *Micrococcus* sp., and *Corynebacterium* sp. or *Microbacterium* sp. were less than 10 cfu/mL on TSA after treatment by 10–30 mg/L SAHW. Treatment by 30 mg/L SAHW has sufficient bactericidal activity on all the bacterial isolates from fresh lettuce.

Long–term bactericidal effect of SAHW on some bacterial strains

Bactericidal effects of SAHW (0.1–30 mg/L of available chlorine) were determined for 7 days on some bacterial strains that were relatively resistant to SAHW. The changes of viable counts enumerated on TSA of some bacterial strains in SAHW solution with different available chlorine concentration are shown in Fig. 1.

Viable counts of *B. cereus* JCM 2152 decreased immediately after treatment by SAHW above 5 mg/L. Following treatment by 30 mg/L SAHW, viable counts of *B. cereus* JCM 2152 had a 5 log reduction at day 0 and had 2 and 5 log difference compared to 5 and 1 mg/L SAHW, respectively. The viable counts further decreased to below the lower limit of detection after day 1 and day 3 for 30, and 5 mg/L SAHW. In contrast, viable counts of 0.5 mg/L SAHW–treated samples increased by 1 log from day 1 to 7. The bactericidal effect of treatment by 30 mg/L SAHW seems sufficient in reducing the viable counts of *B. cereus* JCM 2152.

Viable counts of *S. aureus* IFO 3060 decreased immediately after treatment by SAHW above 10 mg/L. Following treatment by 30 mg/L SAHW, viable counts of *S. aureus* had more than 7 log reduction at day 0 with 2 and 4 log difference compared to 10 and 5 mg/L SAHW, respectively. The viable counts further decreased below the lower limit of detection after day 1 and day 3 for 10, and 5 mg/L SAHW. The bactericidal effect of treatment by SAHW of more than 10 mg/L seems sufficient in reducing the viable counts of *S. aureus*.

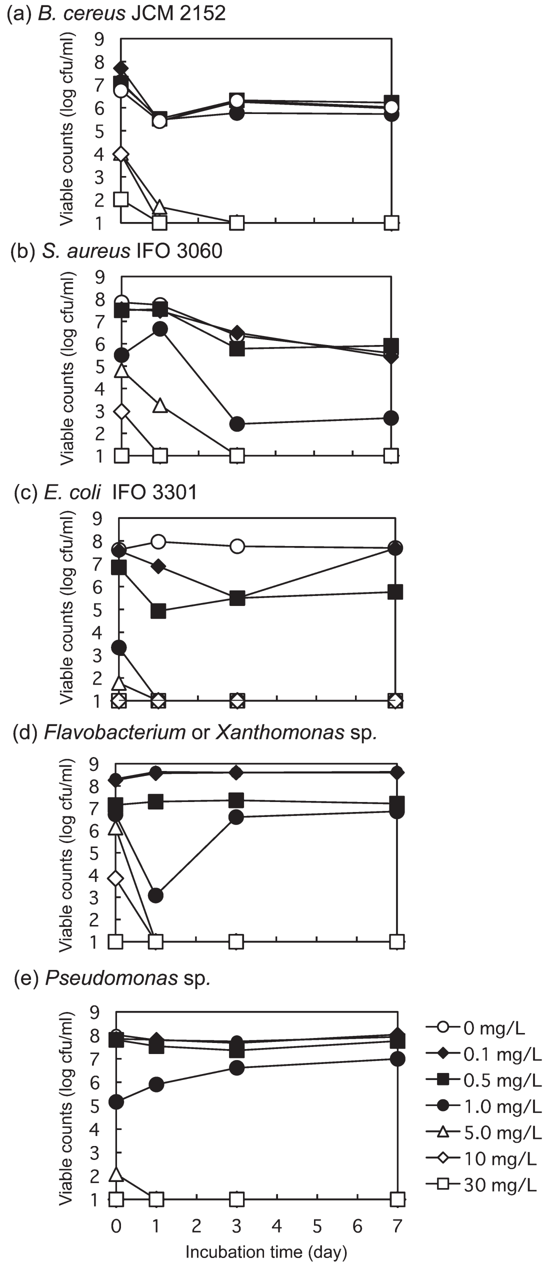

Fig. 1. Long–term bactericidal effect of SAHW on some bacterial strains.
Bactericidal effects of SAHW (0.1–30 mg/L of available chlorine) were determined for 7 days on some bacterial strains that were relatively resistant to SAHW.
Symbols: ○, 0 mg/L; ◆, 0.1 mg/L; ■,0.5 mg/L; ●, 1.0 mg/L; △, 5.0 mg/L; ◇, 10 mg/L; □, 30 mg/L

Viable counts of *E. coli* IFO 3301 decreased immediately after treatment by SAHW above 1 mg/L. Following treatment by 10 mg/L SAHW, viable counts of *E. coli* IFO 3301 had 7 log reduction at day 0 and with 1 and 3 log difference compared to 5 and 1 mg/L SAHW, respectively. The viable counts further decreased below the lower limit of detection after day 1 for 1 and 5 mg/L SAHW. In contrast, viable counts of 0.1 mg/L SAHW–treated cells decreased to 5.5 log cfu/mL at day 3 and increased by 2.5 log from day 3 to 7 suggesting that the recovery of the injured cells were generated in the presence of SAHW at the sub–lethal concentration. The bactericidal effect of treatment by 30 mg/L SAHW seems sufficient in reducing the viable counts of *E. coli* IFO 3301.

Viable counts of *Flavobacterium* or *Xanthomonas* sp. decreased by 7 log immediately after treatment by SAHW at 30 mg/L. Following treatments by 5 and 10 mg/L SAHW, viable counts immediately decreased to 6 and 4 log cfu/mL respectively, further reduction to less than the lower detection limit at day 1, and did not increase at day 7. In the presence of 1 mg/L SAHW, viable counts decreased by 5 log but increased by 4 log at day 3, indicating the recovery of the injured cells in the presence of SAHW at the sub–lethal concentration. The bactericidal effect of treatment by 30 mg/L SAHW seems sufficient in reducing the viable counts of *Flavobacterium* or *Xanthomonas* sp. isolated from lettuce.

Viable counts of *Pseudomonas* sp. decreased immediately after treatment by SAHW above 5 mg/L. Following treatment by 5 mg/L SAHW, viable counts of *Pseudomonas* sp. had 6 log reduction at day 0 with 3 and 6 log difference compared to 1 and 0.5 mg/L SAHW, respectively. The viable counts further decreased to below the lower limit of detection after day 1 for 5 mg/L SAHW. In contrast, viable counts of 1 mg/L SAHW–treated cells immediately decreased to 5 log cfu/mL at day 0 but gradually increased by 2 log from day 0 to 7, suggesting that the recovery of the injured cells were generated in the presence of SAHW at the sub–lethal concentration.

Comparison of bactericidal effect of NaOCl on *Salmonella* Enteritidis

Bactericidal effects of SAHW were compared with those of NaOCl and H_2O_2 on *S*. Enteritidis. Figure 2 shows the changes of viable counts of *S*. Enteritidis after suspension in NaOCl, H_2O_2 and SAHW at various concentrations.

Viable counts of *S*. Enteritidis decreased immediately after treatment by NaOCl, however, recovery of the culturability of *S*. Enteritidis was evident after day 1 and 2 for 0.5 and 0.75 mg/L NaOCl, respectively. There was a 5.5 log increase in 0.75 mg/L NaOCl–treated cells from day 2 to day 5 compared to a 2 log cfu/mL increase of cells treated with 0.5 mg/L NaOCl.

Bactericidal effect of H_2O_2 (1000–2000 mg/L) was determined on *S*. Enteritidis for 5 days. The initial viable counts of all the H_2O_2–treated cell suspensions of *S*. Enteritidis was about 8 log cfu/mL. The counts slowly decreased over time in the order of 2000, 1750, 1250, and 1000 mg/L. After day 2, counts of 2000 mg/L, and 1750 mg/L H_2O_2–treated cells were less than 10 cfu/mL, the lower limit of detection. Treatment by 1750 to 2000 mg/L H_2O_2 has sufficient bactericidal effect on *S*. Enteritidis cells, however, at a higher concentration compared to NaOCl or SAHW.

Viable counts of *S*. Enteritidis decreased immediately after treatment by SAHW above 5 mg/L. Higher reduction of viable counts were evident in 5, and 30 mg/L SAHW–treated samples from day 0 to day 7. Compared to NaOCl, there was not much generation of the injured–but–recoverable cells at the sub–lethal SAHW concentration. The bactericidal effect of treatment by 5 to 30 mg/L SAHW seems sufficient in reducing the viable counts of *S*. Enteritidis.

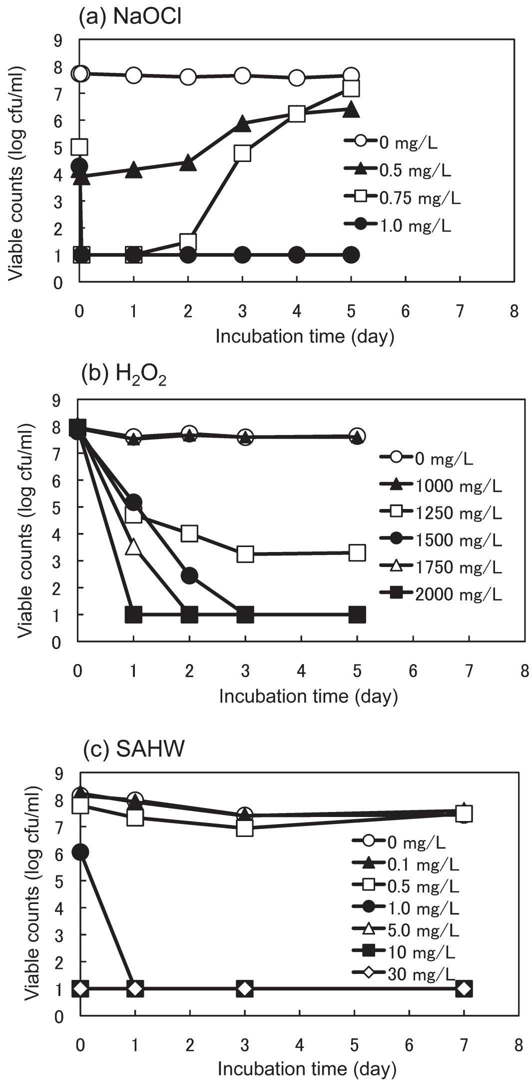

Fig. 2. Changes of viable counts of *S*. Enteritidis incubated in NaOCl, H_2O_2, and SAHW at various concentrations.
S. Enteritidis cells were suspended in 10 mL solution of (a) NaOCl (0.5–1.0 mg/L), (b) H_2O_2 (1000–2000 mg/L), or (c) SAHW (0.1–30 mg/L of available chlorine concentration) at a final cell concentration of 10^7–10^8 cfu/mL. The cell suspensions were kept at 25 °C for 5 or 7 days. Aliquots of the suspensions were withdrawn at suitable intervals, and viable counts were determined by the conventional plating method using TSA. Symbols are indicated in each figure.

DISCUSSION

Pathogenic bacteria can contaminate raw agricultural produce through various pathways; therefore there is a need for effective decontamination of fresh produce. Decontamination of produce plays an important role in the preservation of food quality and safety of consumption (Nguyen–The and Carlin, 1992). Unfortunately, the 'fresh' nature of produce restricts the use of thermal decontamination (Soli *et al*., 2010). Washing produce with tap water cannot completely remove pathogenic and naturally occurring bacteria, therefore, promoting the development of new non–thermal sterilizers such as electrolyzed water to eliminate pathogenic bacteria on fresh produce (Guentzel *et al*., 2008). Among electrolyzed water, SAHW has attracted attention in regard to washing and sanitizing treatments for the removal or inactivation of pathogens on food.

Our study showed that SAHW (30 mg/L of available chlorine) has sufficient bactericidal effect on pure cultures of some Gram–negative (*S*. Enteritidis, *E. coli*) and Gram–positive (*S. aureus, L. monocytogenes, B. cereus*) pathogenic bacteria as well as on some bacterial strains (*Flavobacterium* or X*anthomonas* sp., *Pseudomonas* sp., *Kurthia* sp., *Micrococcus* sp., *Corynebacterium* or *Microbacterium* sp.) isolated from fresh lettuce (Tables 1 and 2). Viable counts of all tested bacteria decreased immediately after treatment by SAHW.

Counts of all the pathogenic bacteria decreased by 1–5 log after treatment by 1 mg/L SAHW and further decreased as the available chlorine concentration of SAHW was increased to 30 mg/L. Most bacterial cells with the exception of *B. cereus*, and *S. aureus* were not culturable on TSA after treatment by 10 to 30 mg/L SAHW. Although *B. cereus* JCM 2152 had counts of 1.0×10^2 cfu/mL after treatment by 30 mg/L SAHW, the counts further decreased to below the lower limit of detection (10 cfu/mL) after day 1. Population of all the bacterial isolates from lettuce decreased by 2–3 log after treatment by 1 mg/L SAHW and further decreased as the chlorine concentration of SAHW was increased to 30 mg/L. Predominant bacteria in fresh lettuce such as *Pseudomonas* sp., *Flavobacterium* or *Xanthomonas* sp., *Kurthia* sp., *Micrococcus* sp., and *Corynebacterium* or *Microbacterium* sp. were not culturable on TSA after treatment by 30 mg/L SAHW. SAHW also had sufficient bactericidal activity on *B. cereus* (diarrheal, emetic), and *E. coli* O157:H7 immediately after the treatment at 30 mg/L (Table 1). Following treatment by 30 mg/L SAHW, viable counts of *B. cereus* (diarrheal) decreased by 5 log cfu/mL (data not shown). The counts further decreased to less than the lower limit of detection after day 1 similar to *B. cereus* JCM 2152 (Fig. 1). After treatment by 30 mg/L SAHW, viable counts of *B. cereus* (emetic) had a 7 log cfu/mL reduction at day 0. Treatment by 30 mg/L SAHW was very effective on *B. cereus* (emetic) from day 0 to day 7 maintaining viable counts less than 1 log cfu/mL (data not shown). Viable counts of *E. coli* O157:H7 decreased immediately after treatment by SAHW (Table 1). Bactericidal activity of 5 mg/L SAHW was potent on *E. coli* O157:H7 than *E. coli* IFO 3301 from day 0 to day 7 maintaining viable counts less than 1 log cfu/mL (data not shown). Although the concentration of SAHW in the surrounding environment of some Gram–negative bacteria in food is sub–lethal such as lower than 1 mg/L, some cells became injured–but–recoverable and recovered culturability after incubation for a few days (Fig. 1). However, the level of recovery seemed lower than that of the injured–but–recoverable cells generated in the presence of NaOCl (Fig. 2). These results indicated that long–term SAHW treatment above 5 mg/L is effective on reducing both viable and pathogenic bacterial counts in food and food–processing environment.

SAHW has better bactericidal effects on *S*. Enteritidis after 5 days as compared to conventional sterilizers, NaOCl and H_2O_2 solution (Fig. 2). The main chlorine sanitizer that has long been recognized and has a high disinfection efficacy in the food industry is NaOCl (100–200 mg/L) (Dychdala, 2001; Takano and Yokoyama, 2001). In our study, viable counts of *S*. Enteritidis decreased immediately after treatment with NaOCl, however, recovery of culturability of *S*. Enteritidis was evident after day 1 for both 0.5, and 0.75 mg/L NaOCl. There was a further 2 to 5 log increase of viable counts for NaOCl–treated samples from day 2 to day 5, thus, 0.5 to 0.75 mg/L NaOCl is not sufficient in reducing viable counts of *S*. Enteritidis even though the level of concentration was manageable. It is well known in the food industry that if the concentration of NaOCl in the surrounding environment of Gram–negative bacteria in food is sub–lethal, the cells that were injured–but–recoverable will show regrowths after incubation for a few days. H_2O_2 is currently classified as GRAS (generally recognized as safe) for use in food products but has not yet been approved as an antimicrobial wash for produce (Sapers, 2001). Acceptable uses include that of bleaching agent, oxidizing and reducing agent, and antimicrobial. Lin *et al*. (2002) reported that the treatment of lettuce with 20000 mg/L (2%) H_2O_2 at 50 ℃ reduced *E. coli* O157:H7 and *L. monocytogenes* by 4 and 3 logs respectively. In our study, initial counts (8 log cfu/mL) of *S*. Enteritidis after H_2O_2 treatment slowly decreased over time in the order of 2000, 1750, 1250, and 1000 mg/L (Fig.2). Treatment by 1750 to 2000 mg/L H_2O_2 has sufficient bactericidal effect on day 2 and 1, respectively on *S*. Enteritidis cells, however, at a higher concentration compared to NaOCl or SAHW. The results indicated that longer treatment time is required for effective decontamination of bacteria by H_2O_2. On the other hand, SAHW–treated *S*. Enteritidis immediately decreased with higher reduction counts in 1, 5, and 30 mg/L available chlorine concentation and there were few injured–but–recoverable cells from day 0 to day 7 unlike NaOCl and H_2O_2.

Okamoto *et al*. (2006) reported that the important characteristic of SlAEW is the potential to lower the residual available chlorine in vegetables after the disinfection treatment compared to other chlorine sanitizers while Koide *et al*. (2009) reported that SlAEW would reduce its available chlorine when exposed to light and air. Since the available chlorine concentration of SlAEW or SAHW in our case, is originally low (10–30 mg/L), they suggested to having a constant flow rate of SlAEW by the electrolysis apparatus during the treatment to stabilize the available chlorine level and consequently the disinfection efficacy.

In conclusion, the results of this study indicated that 30 mg/L SAHW effectively reduces the population of *S.* Enteritidis, *E. coli*, *S. aureus*, *L. monocytogenes*, and *B. cereus* as well as bacterial strains of *Flavobacterium* or *Xanthomonas* sp., *Pseudomonas* sp., *Kurthia* sp., *Micrococcus* sp., *Corynebacterium* or *Microbacterium* sp. isolated from fresh lettuce by more than 5 log immediately after treatment. Treatment by 30 mg/L SAHW seems to be a promising non–thermal disinfection method of food and agricultural produce for both Gram–negative and positive bacteria. Moreover, use of SAHW will allow reduction of the amount of free chlorine used for disinfection of fresh–cut produce by the food industry.

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