



## Efficacy of chlorine and peroxyacetic acid on reduction of natural microflora, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* spp. on mung bean sprouts



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### ABSTRACT

Sprouts-related outbreaks have risen due to increased raw sprouts consumption. To minimize such cases, chemical sanitations are applied. While chlorine is commonly used, concerns with its effectiveness and health implication have prompted researchers to seek alternatives. Peroxyacetic acid (PAA) has shown efficacy in inactivating foodborne pathogens on fresh vegetables, and hence could be considered as an alternative. Thus, the objective of this study was to compare the efficacy of chlorine and PAA in inactivating *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., and natural microflora on mung bean sprouts. Resistance of non- and acid-adapted pathogens to these sanitizer treatments was also evaluated. Un-inoculated and inoculated sprouts were treated with chlorine at 106, 130 and 170 ppm and PAA at 25, 51 and 70 ppm for 90 and 180 s at room temperature. Overall, the greater log reductions were obtained with the increase in the sanitizer concentration. For 180 s, chlorine treatment at 170 ppm reduced 2.0, 1.3, 1.5, 0.9-log and PAA treatment at 70 ppm resulted in 2.3, 1.8, 2.1, 1.1-log reductions for non-adapted *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* spp., and natural microflora, respectively. These results revealed that the efficacy of PAA was significantly better than or similar to that of chlorine. For acid-adapted cells, these sanitizer treatments were less effective with the ranges of 1.0–1.2-log reductions for chlorine and 1.1–1.6-log reductions for PAA compared to non-adapted cells, indicating that acid-adapted cells were more resistant to the sanitizing treatment. These data suggest that PAA may replace chlorine in the disinfection of mung bean sprouts and that acid-adapted pathogens should be used to design an effective sanitizing strategy.

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### 1. Introduction

In the last few decades, seed sprouts have been gaining much popularity amongst consumers. However, the high nutritional value of this fresh produce serves as a perfect source for the growth of foodborne microorganisms (Waje et al., 2009). In addition, the seed sprouts are germinated under warm and humid conditions which are ideal and optimal for bacterial proliferation (Taormina et al., 1999). With the shift towards an increased consumption of raw sprouts, there is also a corresponding rise in sprouts-related foodborne diseases (Health Canada, 2011). Several foodborne pathogens including *Bacillus cereus*, *Escherichia coli* O157:H7 and *Salmonella* spp. have

been identified as main causative agents for sprouts-associated foodborne outbreaks (Waje et al., 2009; Singla et al., 2011). Although no known sprouts-associated outbreaks were associated with *Listeria monocytogenes*, there have been reports on sprouts recall due to detected *L. monocytogenes* (US FDA, 2012). The presence of these foodborne pathogens on the sprouts can be attributed to either the use of contaminated seeds or post-harvest contamination.

Unlike the western countries, the Asian countries do not consume much raw sprouts, such as alfalfa and clover sprouts. Instead, the mung bean sprouts (also known as bean sprouts or taogey) are more widely consumed in the region. Depending on the country and various ethnic cultures, these bean sprouts may be consumed raw, blanched or stir-fried slightly (Hutton, 2007). With respect to the Singapore market, bean sprouts are grown locally and supplemented mainly through the imports from neighboring countries such as Malaysia. Some studies in the Southeast Asia

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(SEA) region have been conducted to analyze the microbiological quality in bean sprouts and showed that they have high microbial load including aerobic bacteria, coliforms, and yeast and molds (Gabriel et al., 2007; Seow et al., 2012). In particular, the studies conducted in Malaysia and Philippines revealed that the bean sprouts were positive for *E. coli* O157:H7 and *Salmonella* spp (Arumugaswamy et al., 1995; Gabriel et al., 2007).

The sprout industry has mitigated the risk of foodborne illnesses through the use of chemical sanitizers, irradiation and heat treatment (Taormina et al., 1999). Amongst these, the use of chemical sanitizers to reduce microbiological load on the sprouts has been preferred since these are more economically feasible than other technologies. Among the chemical sanitizers used, chlorine is the most popular and traditional sanitizer which has been used to disinfect fresh produce (Beuchat, 1998). However, several drawbacks of chlorine treatment on fresh produce had been identified. Chlorine is highly corrosive and may form carcinogens when reaction between chlorine and organic matters take place, particularly at high concentrations (Rodgers et al., 2004). Furthermore, the study conducted by Singh et al. (2005) has shown that only 1.6-log reduction was achieved when 20,000 ppm calcium chloride was directly treated to wheat sprouts for 15 min, indicating the inadequacies of chlorine treatment. Therefore, an alternative sanitizer should be explored to increase efficiency of inactivation of pathogenic bacteria on bean sprouts without health impacts.

Peroxyacetic acid (PAA) is one of the alternative sanitizers that have been studied. PAA is an approved fresh produce sanitizer by the United States (US) Food and Drug Administration (FDA) (Ruiz-Cruz et al., 2007). The disinfection efficacy of PAA is not subjected to pH fluctuations as that of chlorine (Buchholz and Matthews, 2010). In addition, the activity of PAA does not weaken as much as chlorine in the presence of organic matter (Small et al., 2007; Vandekinderen et al., 2009). PAA has also lesser detrimental impacts on human health and environment as compared to chlorine. There is no formation of harmful chlorinated by-products (Kitsis, 2004) and excess PAA is broken down to acetic acid and oxygen (Monarca et al., 2002). Hence, PAA has the potential to replace chlorine in disinfecting bean sprouts.

Bacteria may be exposed to acidic environments when they are present in acidic soil or water, thus it is possible to contaminate sprouts with these acid-adapted pathogens during pre-harvesting. These acid-adapted cells have been reported to become tolerant to processing operations such as heat, salt, disinfectants and irradiation (Goodson and Rowbury, 1989; Leyer and Johnson, 1993; Lin et al., 2011). Hence, it is worthy to test acid-adapted cells to see if they are more resistant to the sanitizer treatments than normal cells.

Although comparison of effectiveness of PAA and calcium hypochlorite had been performed on alfalfa sprout seeds (Buchholz and Matthews, 2010), this has yet to be done on mung bean sprouts which are commonly eaten in Asia. Therefore, the aim of this study was to determine if PAA can be used to replace chlorine on the sanitization of mung bean sprouts. To do so, the efficacy of these sanitizers was evaluated and compared based on the reduction of natural microflora and common sprouts-associated foodborne pathogens such as *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* spp. The resistance of non-adapted and acid-adapted pathogens to these sanitizers was also compared.

## 2. Materials and methods

### 2.1. Bacterial culture

*E. coli* O157:H7 (EDL 933) was obtained from Dr. Henry Mok at Department of Biological Science in the National University of

Singapore. *Listeria monocytogenes* serovar 1/2a (BAA-679) and five *Salmonella* serotypes (*S. Montevideo* BAA 710, *S. Newport* BAA 707, *S. Saintpaul* ATCC 9712, *S. Typhimurium* ATCC 14028 and *S. Tennessee* ATCC 10722) were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). *E. coli* O157:H7, *L. monocytogenes* 1/2a and *Salmonella* serovars were adapted to 100 µg/ml nalidixic acid (Sigma–Aldrich, St Louis, MO, USA) by stepwise increment of nalidixic acid after each transfer of the respective culture. All media used in this study were supplemented with 100 µg/ml nalidixic acid so that these pathogens isolated from inoculated bean sprouts were relatively free from other background bacterial contaminants. Prior to inoculation, the respective pathogenic strains were cultivated in sterile tryptic soy broth (TSB; Acumedia, Lansing, MI, USA) containing nalidixic acid (TSBN) at 37 °C for 24 h with two consecutive transfers. After 24-h of incubation, 1 ml of the individual culture was centrifuged (3500 g for 10 min, 4 °C) and washed with 1 ml of sterile 0.1% peptone water (Oxoid, Hampshire, UK). The re-suspended pellets were centrifuged (3500 g for 10 min, 4 °C). The washing and centrifugation steps were repeated twice. The individual harvested pellets were re-suspended in 1 ml of sterile 0.1% peptone water to obtain a final cell density of 10<sup>8</sup> CFU/ml. It is known that the effectiveness of sanitizer is strain dependent (Hora et al., 2007), thus the five *Salmonella* serovars were aseptically combined to produce a cocktail after washing. On the other hand, only single strain of *E. coli* O157:H7 or *L. monocytogenes* was used in this study since it was difficult to obtain these two pathogens in Singapore and from other countries. *E. coli* O157:H7, *L. monocytogenes* or *Salmonella* cocktail was suspended in 1 L of distilled water to inoculate bean sprouts.

### 2.2. Preparation of acid-adapted cells

Acid-adapted cells were prepared by transferring 1 ml of respective nalidixic acid-adapted bacterial strain into 9 ml of TSBN supplemented with 1% glucose (Sinopharm chemical reagent, Shanghai, China) (TSBN-G) and incubated at 37 °C for 24 h with two consecutive transfers prior to inoculation (Beuchat and Mann, 2008). Before inoculation on mung bean sprouts, acid-adapted cells were washed as described above.

### 2.3. Inoculation

Mung bean sprouts were purchased from a local farm and unwashed bean sprouts were stored at 4 °C during experiments with a maximum shelf life of 5 days. Prior to inoculation, the bean sprouts were removed from the refrigerator and left at room temperature (25 ± 2 °C) for 45 min. Approximately 450-g of sprouts was submerged in 1 L of prepared *E. coli* O157:H7, *L. monocytogenes* or *Salmonella* cocktail (ca. 10<sup>5</sup>–10<sup>6</sup> CFU/ml) suspension for 45 min. A sterile magnetic stirrer was added into the suspension to ensure even inoculation. The inoculated sprouts were dried on sterile plastic trays in a biosafety cabinet for 3–4 h.

### 2.4. Preparation of sanitizers

Sodium hypochlorite, commonly known as chlorine (XY-12; Ecolab, St Paul, MN, USA) and peroxyacetic acid (PAA) (Oxonia Active; Ecolab) were used in this study. Since the maximum concentrations of chlorine and PAA are 200 and 80 ppm for fresh produce, respectively (Ruiz-Cruz et al., 2007) and the sprout industry in Singapore also prefers to use lower concentrations than their maximum concentrations, the following concentrations of each sanitizer were prepared; chlorine (106, 130 and 170 ppm) and PAA (25, 51 and 70 ppm) by diluting the concentrated solution with sterile distilled water according to manufacturer's direction. The

concentrations of free chlorine and total PAA were determined using RQflex® 10 Reflectoquant® (Merck, Darmstadt, Germany) according to manufacturer's direction.

### 2.5. Sanitizer treatment

Sanitizer treatments were conducted to both un-inoculated and inoculated mung bean sprouts. The bean sprouts were weighed and divided into sets of 100 g. A 100-g of bean sprouts was transferred into 1 L of prepared sanitizer solution and treated for 90 or 180 s at room temperature with stirring which may provide uniform sanitizing. The bean sprouts were also washed with sterile distilled water without sanitizer for 90 or 180 s, which were served as controls. Visual verification was done to ensure that the bean sprouts were completely immersed on the sanitizers throughout the sanitizer treatment.

### 2.6. Microbiological analysis

At 90 s or 180 s, the treated bean sprouts (10-g each) were transferred into a sterile stomacher bag containing 90 ml of Dey/Engley (D/E) neutralizing broth (Acumedia) to neutralize the presence of any residual sanitizers and homogenized using a stomacher (IUL Instruments, Barcelona, Spain) for 1 min. The homogenate was serially diluted using 0.1% sterile peptone water and pour plated on sterile tryptic soy agar (TSA; Acumedia) or TSA containing 100 µg/ml nalidixic acid at 37 °C for 24–48 h. Surviving pathogens were enumerated by manual counting of colonies.

### 2.7. Statistical analysis

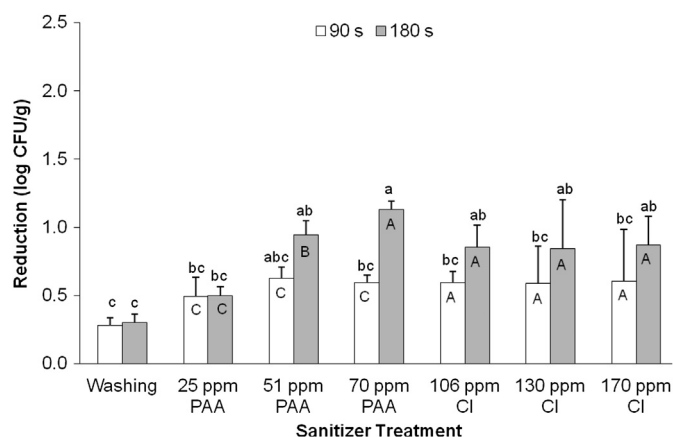
Mean values of bacterial counts were obtained from independent triplicate with duplicated sampling ( $n = 6$ ). All data were statistically analyzed by ANOVA using the SPSS® statistical software (IBM, Armonk, NY, USA). Least significant difference (LSD) was used to compare the mean values at 5% significance level ( $P < 0.05$ ).

## 3. Results

The initial microbial load of un-inoculated mung bean sprouts was approximately  $10^7$  to  $10^8$  CFU/g. The bean sprouts were treated with different concentrations of PAA and chlorine solutions for 90 or 180 s. Water washing decreased natural microflora and inoculated pathogens by less than 0.5-log unit (Figs. 1–4). For natural microflora, peroxyacetic acid (PAA) treatments resulted in 0.5–1.1-log unit reductions and the efficacy was significantly ( $P < 0.05$ ) enhanced with an increase in PAA concentrations and exposure time (Fig. 1). Chlorine treatments reduced 0.6 to 0.9-log of the population of natural microflora, but the concentration of chlorine and length of an exposure time did not affect the overall efficiency of the sanitation treatment. There was no significant ( $P > 0.05$ ) difference in reducing natural microflora on bean sprouts between PAA and chlorine treatments.

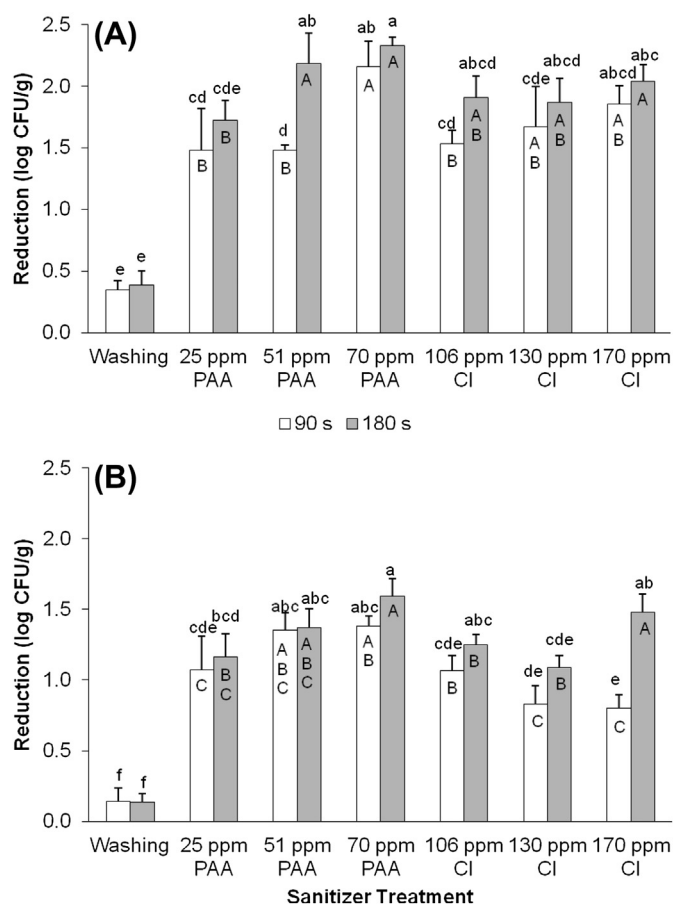
A total of 1.5–2.3-logs and 1.5 to 2.0-logs in the populations of non-adapted *E. coli* O157:H7 was reduced by PAA and chlorine treatments, respectively, showing approximately 1.0–2.0 further reduction compared with water washing (Fig. 2A). There was no significant ( $P > 0.05$ ) difference between 90 and 180 s, except for PAA treatment at 51 ppm. Higher concentrations of PAA delivered more reduction, while no difference was observed among three chlorine concentrations. The efficacy of PAA treatment did not significantly ( $P > 0.05$ ) differ from chlorine treatment at the highest concentration.

For acid-adapted *E. coli* O157:H7, PAA treatments resulted in 1.0 to 1.6-log reductions and chlorine treatments caused 0.8 to 1.5-log

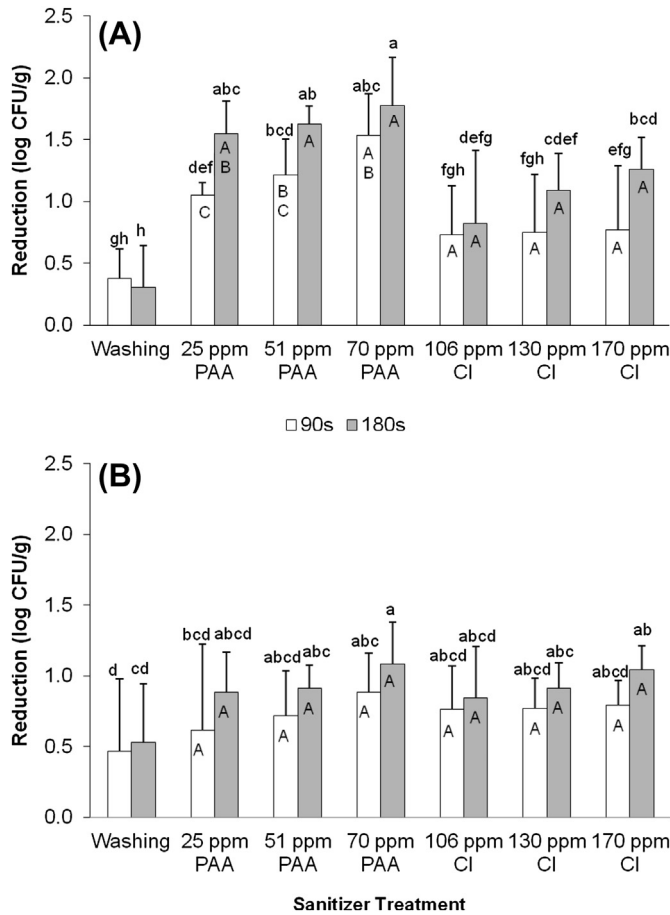


**Fig. 1.** Efficacy of peroxyacetic acid (PAA) and chlorine (Cl) in reducing natural microflora on the mung bean sprouts for 90 s and 180 s. Different letters (ab) across the sanitizer treatments indicate a significant ( $P < 0.05$ ) difference in the log reductions. Different letters (AB) indicate a significant ( $P < 0.05$ ) difference in the log reductions within PAA or chlorine treatment, respectively.

reduction, showing no significant ( $P > 0.05$ ) difference between them (Fig. 2B). It was observed that an increase in the PAA concentration enhanced the overall efficacy of the disinfection treatments, while an increase in exposure time to PAA treatment did not affect on the log reductions. Unlike PAA, the efficacy of chlorine treatment was significantly ( $P < 0.05$ ) affected by both



**Fig. 2.** Efficacy of peroxyacetic acid (PAA) and chlorine (Cl) in reducing non-adapted (A) and acid-adapted (B) *Escherichia coli* O157:H7 on the mung bean sprouts for 90 s and 180 s.



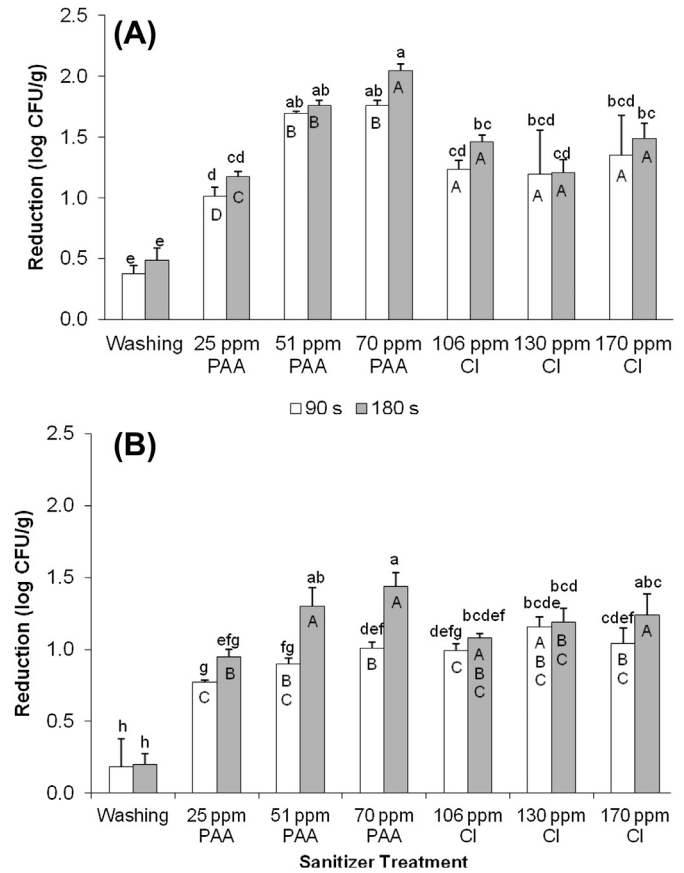
**Fig. 3.** Efficacy of peroxyacetic acid (PAA) and chlorine (Cl) in reducing non-adapted (A) and acid-adapted (B) *Listeria monocytogenes* on the mung bean sprouts for 90 s and 180 s.

concentration and treatment time. The maximum log reductions were obtained at the highest concentrations of PAA and chlorine for 180 s but there was no significant difference between the two sanitizer treatments.

Treatment of the bean sprouts with PAA caused 1.0–1.8 log reductions in non-adapted *L. monocytogenes* levels, while chlorine treatment resulted in 0.7–1.3 log reductions (Fig. 3A). The greatest reduction was obtained with treatment of PAA at 70 ppm for 180 s, which significantly ( $P < 0.05$ ) differed from chlorine treatment. For chlorine treatment, prolonged exposure time and increased concentration did not affect on the inactivation of *L. monocytogenes* on the bean sprouts, whereas the efficacy of PAA was slightly influenced by treatment time but not its concentration.

Unlike non-adapted *L. monocytogenes*, only the highest concentrations of PAA and chlorine were effective in eliminating acid-adapted *L. monocytogenes* on mung bean sprouts, showing additional 0.5-log reductions compared with water washing (Fig. 3B). In addition, the efficacy of PAA treatment did not differ with chlorine treatment at the highest concentration. Exposure time and concentrations of PAA and chlorine failed to show any significant ( $P > 0.05$ ) difference in reducing bacterial cells.

For non-adapted *Salmonella* spp., about 1.0–2.1-log reductions were achieved by PAA treatments, exhibiting that 70 ppm PAA for 180 s caused the highest log reduction (Fig. 4A). Higher concentrations and longer treatment time improved the efficacy of PAA. Chlorine treatments resulted in 1.2 to 1.5-log reductions and both the chlorine concentration and exposure time did not significantly ( $P > 0.05$ ) affect the overall log reductions. A comparison between



**Fig. 4.** Efficacy of peroxyacetic acid (PAA) and chlorine (Cl) in reducing non-adapted (A) and acid-adapted (B) *Salmonella* spp. on the mung bean sprouts for 90 s and 180 s.

two disinfection treatments showed that PAA was significantly ( $P < 0.05$ ) more effective than chlorine in the elimination of *Salmonella* spp on the mung bean sprouts.

PAA treatments led to 0.8 to 1.4-log reductions of acid-adapted *Salmonella* spp and approximately 1.0 to 1.2-log unit of the population was inactivated by chlorine treatment (Fig. 4B). It was observed that both the PAA concentration and exposure time did have a significant ( $P < 0.05$ ) impact on the overall efficacy of the treatment, while these factors did not affect the efficacy of chlorine treatment. There was no significant ( $P > 0.05$ ) difference between the effectiveness of PAA and chlorine treatments in removing bacterial cells on bean sprouts.

The effectiveness of PAA and chlorine treatments in inactivating non-adapted and acid-adapted cells was compared (Table 1).

**Table 1**

Comparison of reduction (log CFU/g) of non-adapted (NA) and acid-adapted (AA) pathogens on the mung bean sprouts by peroxyacetic acid (PAA) and chlorine (Cl) treatments at the highest concentrations for 180 s.

Bacterial strain		Reduction (log CFU/g)	
		PAA at 70 ppm	Cl at 170 ppm
<i>E. coli</i> O157:H7	NA	2.3 ± 0.1 <sup>a</sup>	2.0 ± 0.1 <sup>a</sup>
	AA	1.6 ± 0.1 <sup>b</sup>	1.5 ± 0.1 <sup>b</sup>
<i>L. monocytogenes</i>	NA	1.8 ± 0.4 <sup>a</sup>	1.3 ± 0.3 <sup>a</sup>
	AA	0.1 ± 0.3 <sup>b</sup>	1.0 ± 0.2 <sup>b</sup>
<i>Salmonella</i> spp.	NA	2.1 ± 0.1 <sup>a</sup>	1.5 ± 0.1 <sup>a</sup>
	AA	1.4 ± 0.1 <sup>b</sup>	1.2 ± 0.1 <sup>b</sup>

Values are mean ± SD of 3 replications. Different letters (<sup>ab</sup>) between NA and AA within each bacterial strain and each sanitizer indicate a significant ( $P < 0.05$ ) difference.



Regardless of bacterial strain, acid-adapted cells were about 1.5- and 1.3-time more resistant to PAA and chlorine treatment, respectively, indicating that acid adaptation could protect bacterial cells from these sanitizer treatments.

#### 4. Discussion

This study was conducted to compare the efficacy of PAA and chlorine in eliminating bacterial contaminants including natural microflora and foodborne pathogens on mung bean sprouts to see if PAA can replace chlorine. For natural microflora, a maximum of 1.0-log reduction was observed in the bean sprouts after treatments with these two sanitizers, showing lesser reduction than inoculated pathogens in this study. Similarly, Splittstoesser et al. (1983) showed that 100 ppm chlorine reduced less than 1.0-log unit of the natural microflora on sprouting mung beans. Vandekinderen et al. (2009) reported that less than 80 ppm PAA treatments resulted in a minimum of 0.5-log unit reductions of the natural microflora on carrots, cabbage, iceberg lettuce and leek. These results indicated that natural microflora was more resistant to the applied sanitation treatments as compared to the inoculated pathogens. Such resistance might be due to the strength of bacterial attachment to the bean sprouts since the natural microflora have sufficient time to strongly adhere onto the surface of the sprouts during the germination process. Sapers (2001) reported that approximately 3-log reduction and less than 1-log reduction for *S. Stanley* on fruits and vegetables was achieved using the same sanitizing treatment right after inoculation and 72 h post-inoculation, demonstrating that the strength of adherence was directly proportional to the length at which the microorganism attached to the surface of the produce. Another possible explanation could be that the natural microflora is attached to the surface of the bean sprouts in the form of biofilm. It is known that bacteria within the biofilm were more resistant against the actions of sanitizers compared to planktonic bacteria cells (Davidson and Harrison, 2002; Marriott and Gravani, 2006).

Treatments with PAA at 70 ppm and chlorine at 170 ppm for 180 s reduced the inoculated pathogens on the bean sprouts by 1.5–2.3 log units. Similar results were also found in the previous studies which tested different sprouts or sanitizers. Lee et al. (2002) reported that 200 ppm chlorine treatment decreased *S. typhimurium* and *L. monocytogenes* by 2.23- and 1.02-log units, respectively, on mung bean sprouts. The study conducted by Jin and Lee (2007) showed that there were 3.0 and 1.5-log reductions of *S. typhimurium* and *L. monocytogenes*, respectively, when inoculated mung bean sprouts were treated with 100 ppm chlorine dioxide. The populations of *E. coli* O157:H7, *L. monocytogenes* and *S. Typhimurium* on broccoli sprouts were reduced by 1.66-, 1.24-, 1.64-log as a result of treatment with 50 ppm chlorine dioxide (Kim et al., 2009).

In this study, PAA treatment was slightly more or equivalently effective than or to chlorine treatment in inactivating inoculated foodborne pathogens on mung bean sprouts. A similar result was observed in the study conducted by Yuk et al. (2005) which showed that 87 ppm PAA and 200 ppm chlorine led to approximately 2.72 and 2.53-log reductions on *Salmonella* spp. on the stem scar of tomatoes. Contrarily, Rodgers et al. (2004) reported 200 ppm chlorine to be more effective than 80 ppm PAA in the reduction of *E. coli* O157:H7 on apples, lettuce, strawberries and cantaloupe. These contradictory results indicate that the effectiveness of sanitizers might be dependent on not only bacterial strains but also the tested commodities which have different surface characteristics. Yuk et al. (2006) have shown that the chlorine treatment at 200 ppm was effective in eliminating *Salmonella* spp. on the surface of cucumber but not bell pepper.

The influence of the sanitizer concentrations and treatment time for sanitation was evaluated in this study to determine

whether these two factors affect the effectiveness of sanitizers. PAA treatment showed that its bactericidal effect was significantly enhanced with increased concentrations but about 60 ppm difference in the concentrations of chlorine had no apparent effect on its effectiveness. Similarly, Vandekinderen et al. (2009) also confirmed through a mathematical model that the concentration of PAA highly affected the microbial reduction on fresh-cut produce such as carrot, white cabbage and iceberg lettuce. Unlike the present results, Rodgers et al. (2004) reported 200 ppm chlorine to be more effective than 100 ppm chlorine in the elimination of background microorganisms on apples, lettuce, strawberries and cantaloupe.

For exposure time, the present results indicated that an increase in treatment time was less effective in eliminating microorganisms on bean sprouts compared with the effect of sanitizer concentration although there was somewhat variation among bacterial strains with regards to the effect of time. Similar results were also observed in the studies carried out by Yuk et al. (2005, 2006), showing that efficacies of the chlorine and PAA treatments on tomatoes, bell peppers and cucumbers were insignificantly different at 60 s and 120 s. This may be because the elimination of bacteria by the sanitizers follows a dual-phase inactivation which is rapid inactivation of loosely attached bacteria in the first minute, followed by a slower inactivation of bacteria hidden deep within the commodity in the remaining minutes (Vandekinderen et al., 2009). Thus, bacterial inactivation could mostly occur at the initial point of contact, suggesting that a slight increase in exposure time to the sanitizer solution could not significantly improve the efficacy of sanitizers.

This study also compared acid-adapted pathogens with non-adapted control cells to see if there was any difference in their resistance to the sanitizing treatment since it is possible to contaminate with foodborne pathogens which are exposed to the acidic environment. The results showed that acid-adapted cells were more resistant than their non-acid-adapted counterparts when subjected to the same sanitizing treatment. This observation was supported by Lin et al. (2011) who showed that the viability of acid-adapted *S. typhimurium* was higher than non-adapted cells after treatment with chlorine. Contrarily, another study performed by Stopforth et al. (2004) reported that there was no significant difference in the reductions of acid-adapted or non-adapted *E. coli* O157:H7 in wound apples with approximately 200 ppm chlorine treatment, demonstrating that exposure of the non-adapted strain to the moderate acidity of the apple may have acid-shocked the strain to become acid tolerant.

The higher resistance of the acid-adapted pathogens observed from this study may be because acid resistance can impart cross resistance to other environmental stresses (Spector and Kenyon, 2012). Although the molecular and physiological mechanisms has yet to be fully understood, the increased resistance might be due to the changes in the membrane fatty acid composition (Russell, 1995; Casadei et al., 2002; Álvarez-Ordóñez et al., 2009). It is known that bacterial cells can modify their membrane fatty acid composition by increasing the ratio of saturated and cyclopropane fatty acids to unsaturated fatty acids during the adaptation under acidic condition (Brown et al., 1997; Álvarez-Ordóñez et al., 2008, 2009). As a result, highly saturated membrane may confer more resistance against the oxidative damage brought about by PAA and chlorine (Moat et al., 2002). Thus, it should be necessary to use acid-adapted cells in evaluating the efficacy of sanitizers, since the healthy growing cells may not be found in nature.

#### 5. Conclusion

The results showed that the efficacy of PAA was similar or slightly better than that of chlorine in reducing native microflora,

*E. coli* O157:H7, *L. monocytogenes* and *Salmonella* spp. The bactericidal effect of PAA was significantly impacted by its concentration but not exposure time to the sanitizer. Regardless of bacterial strains, acid-adapted cells were more resistant to the sanitizer treatments. Therefore, this study suggests that PAA may serve as an alternative replacement for chlorine and there should be a need of using acid-adapted cells for an accurate evaluation of sanitizers.

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