International Food Research Journal 31(4): 919 - 930 (August 2024)
Journal homepage: *http://www.ifrj.upm.edu.my*

Bacterial control on mung bean and green mustard sprouts by seed sterilisation using electrochemical activated solution before storage and processing

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Article history

Received:
29 July 2023
Received in revised form:
2 June 2024
Accepted:
14 June 2024

Keywords

seed,
sprout,
storage,
mung bean seed,
green mustard seeds,
electrochemical activated solution

Abstract

Sprout is a popular homemade salad, but frequently contaminated with pathogens, which requires efficient disinfection measure. The present work demonstrated an effective method to control microorganisms on seeds before sprout production using electrochemical activated solution (EAS). The seeds were treated with EAS containing different concentrations of chlorine to assess the efficacy of disinfection, germination, and sprout growth. The long-term effect of the treatment with EAS was studied on seeds stored for 4, 8, or 12 w at 8 or 28°C. The results showed that EAS treatment reduced coliform and *Erwinia carotovora* without affecting seed germination or sprout growth. High sterilisation efficacy was achieved at higher available chlorine concentration (ACC) and/or longer treatment. The highest disinfection effect was observed at the ACC of 29.0 ppm and treatment at 15 min. Sterilisation was maintained for 12 w at 8°C or 4 – 8 w at 28°C when the seeds were treated with EAS having 29.0 ppm ACC in 15 min. The present work provided an effective way to control bacteria on seeds, and prevent the transmission of pathogens from seeds to later sprout products, thus improving their safety and hygiene.

DOI
https://doi.org/10.47836/ifrj.31.4.10

Introduction

Raw sprouts are popular in the daily menu of many families around the world (Yogesh and Ali, 2014). They are often consumed as fresh salad without any processing. Unfortunately, fresh salad usually contains pathogens, which can cause serious infection outbreaks (Sakuma *et al*., 2006; Barratt *et al*., 2021). Most of the sprouts are homemade products, not hygiene enough, and easy to spoil due to the lack of effective sterilisation treatments.

The pathogens in the sprouts are usually from seeds rather than contamination during production and food processing. Microorganisms can contaminate the seeds during harvesting and storage (Fu *et al*., 2001; Miyahira and Antunes, 2021). When infected seeds are grown, the microorganisms will also grow simultaneously, and cause diseases to sprouts, and eventually cause health risks to the consumers. Therefore, in sprout production, ensuring the absence of pathogens in seeds is regarded a critical control point, as defined by the Codex Alimentarius Commission (Weiss and Hammes, 2005).

To eliminate pathogens in seeds prior to growing sprouts, sterilisation chemicals have been used (Peñas *et al*., 2009; Yang *et al*., 2013; Smith and Herges, 2018). Chemical agents have a strong antimicrobial effect, but they may contaminate seeds, and reduce seed quality. A previous study showed that the germination percentage of seeds reduced to unacceptable levels due to the chemical treatment (Peñas *et al*., 2009). Several studies have tried the combination of various sterilisation methods, *i.e*.,

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chemical, heat, and ultrasound (Beuchat and Scouten, 2002), or the dry-heat treatment with irradiation (Bari *et al*., 2009). The combination generated confusing results; some resulted in positive effects on reducing bacterial population on seeds, but negatively impacted on the germination or the growth of seeds (Bari *et al*., 2009). Those methods usually use expensive machines, devices, or equipment to sterilise. Therefore, a more robust, cost-effective, and easy-to-apply method is warranted.

Electrochemically activated solution (EAS) produced from diluted salt solution is a strong and benign disinfectant, which has been considered as a promising alternative to conventional sterilisation chemicals (Mohammadi and Ebadi, 2021). When applying direct current to electrodes, diluted salt is electrolysed generating highly oxidative components and radicals such HOCl, ClO_2, H_2O_2, and O_3 (Bialka *et al*., 2004; Mohammadi and Ebadi, 2021). These radicals are strong oxidation agents, which will decompose into NaCl, H_2O, and O_2 during use and storage. Based on previous studies, the slightly acidic solution contains mainly HOCl, which is rapidly decomposed and therefore does not leave any chlorine on disinfection surface (Guentzel *et al*., 2008). The dilute EAS, therefore, can kill bacteria instantly but does not cause any side effect. EAS can be generated as an acidic or alkaline solution, in which pH, oxidation reduction potential (ORP), and available chlorine concentration (ACC) may vary depending on the dilution or the combination of anolyte and catholyte flows (Hai *et al*., 2021). Acidic solution (anolyte solution) usually has pH < 2.7, ORP > 1,000, and ACC from 40 to 90 ppm (Yang *et al*., 2013). When the anolyte is mixed with a catholyte solution, its pH increases, while the ORP and ACC decrease. EAS has been used in the food processing industry due to its low cost and safety to humans and environments (Fabrizio and Cutter, 2004; Bialka *et al*., 2004; Escamilla *et al*., 2019). Recently, the potential use of EAS to control the microbial population in sprout germination and quality has attracted significant attention (Liu and Yu, 2017). Previous studies indicated that the counts of bacteria and moulds in the sprouts were reduced from 1.02 to 1.58 log CFU/g by treating sprouts with EAS (Rui *et al*., 2011; Zhang *et al*., 2016; Liu and Yu, 2017), and the treatment of seeds with EAS before production enhanced the sprout growth (Zhang *et al*., 2016).

Although the potential utilisation of EAS to sterilise and control microbial growth in sprouts has been increasingly investigated, most of the work focused on coliform, yeasts, and moulds. The disinfection activity of EAS against *Erwinia carotovora*, a bacterium that causes rotten disease, has scarcely been examined. It has been proven that seed sterilisation can enhance sprout growth and hygiene. In reality, seeds and other fruits are usually harvested and stored for a certain period (Agrahar-Murugkar and Jha, 2011; Rezaee *et al*., 2013), however, they were only sterilised right before processing. The sterilisation of seeds with EAS before storage for later sprout production has been scarcely investigated. Therefore, the objective of the present work was to treat mung bean and green mustard seeds with EAS, and store them for different times to investigate the effect of the treatment on the population of coliform and *Erwinia carotovora,* seed germination, and sprout growth.

Materials and methods

Materials

The fresh seeds of mung bean (*Vigna radiata* L.) and green mustard (*Brassica juncea* (L.) Czern.) used in the experiments were obtained from VinEco Agricultural Investment, Development and Productions LLC. The uniform seeds were selected and kept at 4°C until used for the experiments. Selected seed samples were artificially infested with coliform (*Enterobacter aerogenes* ATCC 35029, provided by Microbiologics, USA) at around 6 - 7 log CFU/g, or with *Erwinia carotovora* (*E. carotovora* M5.2 provided by Vietnam Academy of Agriculture) at around 5 - 6 log CFU/g before the experiment. The frozen bacteria were taken by a sterile loop, inoculated on an agar plate, and incubated at 37°C for 48 h. The bacteria were then collected and diluted for later experiments.

Electrochemically activated solution preparation

Electrolysis setup including a power supply; an electrolysis chamber having a cathode, anode, and diaphragm; and a quantitative pump to feed KCl solution was developed in the Institute of Environmental Technology, Vietnam Academy of Science and Technology, and was used for EAS production. Typically, an electrochemical solution was generated by continuous electrolysis of KCl solution (1 g/L) at 8 V and 0.7 A in a diaphragm chamber. The KCl solution was continuously supplied to the anode and cathode chambers, with an

anode flow rate of 10 L/h, and a cathode flow rate of 10 L/h, as described previously (Hai *et al*., 2021). EAS was obtained by mixing anode and cathode solution in a volume ratio of 8:0.5. The solution was then diluted with distilled sterilised water at 1.0, 0.8, 0.6, 0.4, and 0.2 strengths to generate the EAS solution with ACC from 5.6 to 29.0 ppm (Table 1).

The pH value and the oxidation reduction potential (ORP) of EAS were determined using the HACH SenSion-156 device. The chlorine available was analysed by an iodometric method (SMEWW 4500- Cl. B) and a photometric method, with HACH DPD reagent (USA) on a DR 2800 equipment (HACH - USA). The properties of EAS solutions are shown in Table 1.

Table 1. Properties of EAS solutions.

Solution	pH	ACC (ppm)	ORP (mV)
Sterilised water (control)	6.7	< 0.05	293
EAS 1.0 strength	6.5	29.0	861
EAS 0.8 strength	6.5	23.1	770
EAS 0.6 strength	6.6	17.5	687
EAS 0.4 strength	6.6	11.8	619
EAS 0.2 strength	6.7	5.6	503

Seed treatments

In a typical experiment, 350 g of green mustard seeds or mung bean seeds were soaked in 1,000 mL beaker containing treating solutions (different EAS or sterilised water as control) for 5, 10, or 15 min, and then treated seeds were separated for later procedures. Immediately, 25 g of treated seeds were used to determine the survival of coliform. The remaining treated seeds were placed on wire screens lined with cheesecloth to dry under a laminar flow hood for 24 h.

Enumeration of total bacterial counts

The method for determining the *E. carotovora* population on seeds was adopted from Hadas *et al*. (2001). Dry seeds (10 g) were ground and then suspended in 45 mL of 0.1% (w/v) saline agar supplemented with 0.05% (w/v) ascorbic acid. The suspension was shaken at 20°C for 2 h, followed by mixing for 15 min in a laboratory blender. A ten-fold series of dilutions was prepared in saline supplemented with 0.05% (w/v) ascorbic acid, followed by plating on crystal violet pectate (CVP) medium (Himedia - M1392), and incubation at 27°C for 3 d. Colonies that produce pits in CVP and with a grey-violet colour were enumerated and expressed as logs of colony-forming unit per gram (log CFU/g).

The coliform counts on the seeds after being treated with different EAS and sterilised water (as a control) were determined according to ISO 4832:2007. All plates were incubated at 37°C for 24 h before coliform colonies were enumerated and expressed as logs of colony forming unit per gram (log CFU/g).

Bacterial growth on treated seeds during storage

The treated seeds (50 g/bag) were stored in plastic bags for 4, 8, or 12 w at 8 or 28°C, corresponding to the refrigeration storage condition or normal storage conditions, respectively. Coliform and *E. carotovora* population in seeds stored under different conditions were then analysed to evaluate the effect of EAS treatment on the bacterial development on seed during storage.

Seed germination and sprout growth

The percentage of seed germination was determined as described by Hu *et al*. (2004). The control and treated seeds (25 g) were placed in sterile hydroponic sponges for 3 d at 25°C. Sterile water was added periodically to maintain a high-moisture environment. The total number of seeds and germinated seeds left in the containers were then counted, where the percentage of germination was defined as the ratio of seeds that germinated over the total number of seeds.

To determine the growth of the sprouts, the germinated seeds in each experimental sample and control were left to continue growing for 4 d under the same cultivation conditions. The length (cm) of the green mustard and mung bean sprouts was measured by a ruler.

Statistical analysis

All trials were replicated three times under the same experimental conditions. Data represent the mean values obtained from three individual trials with two samples. Data were subjected to variance analysis using the Microsoft Excel 2019 program. Significant differences in plate count data were established by the smallest difference at the 5% significance level.

Results and discussion

Effect of EAS treatment on coliform and Erwinia carotovora on seeds

The effect of EAS on coliform counts on seeds is presented in Figure 1. The coliform counts on the green mustard seeds without treatment (control) were 6.2 log CFU/g. It decreased by about 1.30 - 1.38 log CFU/g after treating with water for 5 min, and the decrease was unchanged after longer treatment with water. A similar trend was observed when mung bean seeds was treated with water. Coliform counts were 7.43 log CFU/g without treatment, which decreased to 5.5 - 5.83 log CFU/g after 5 min of water treatment. However, the decrease was significant when the seeds were treated with EAS. Green mustard seed after being treated with different EAS strength for 5, 10, and 10 min produced a sharp decrease in coliform; from 6.20 to below 2.20 log CFU/g. The shortest treatment time (5 min) with the most dilute EAS (5.6 ppm ACC) resulted in a 4 log CFU/g reduction. The higher chlorine concentration, 11.8 to 29.0 ppm corresponding to the strength of 0.4 to 1.0, respectively, generated a higher decrease (5.00 to 5.10 log CFU/g). These results revealed that no considerable improvement in disinfection efficacy was achieved by increasing ACC to more than 11.8 ppm at 5 min of treatment. It was, however, clearly differentiated at the longer time. Treatment in 10 min caused a decrease of 4.44 to 5.54 log CFU/g with EAS containing 5.6 to 29.0 ppm ACC, respectively. Interestingly, the disinfection efficacy reached 100% when green mustard seeds were treated for 15 min with EAS having an ACC of 23.1 ppm and above.

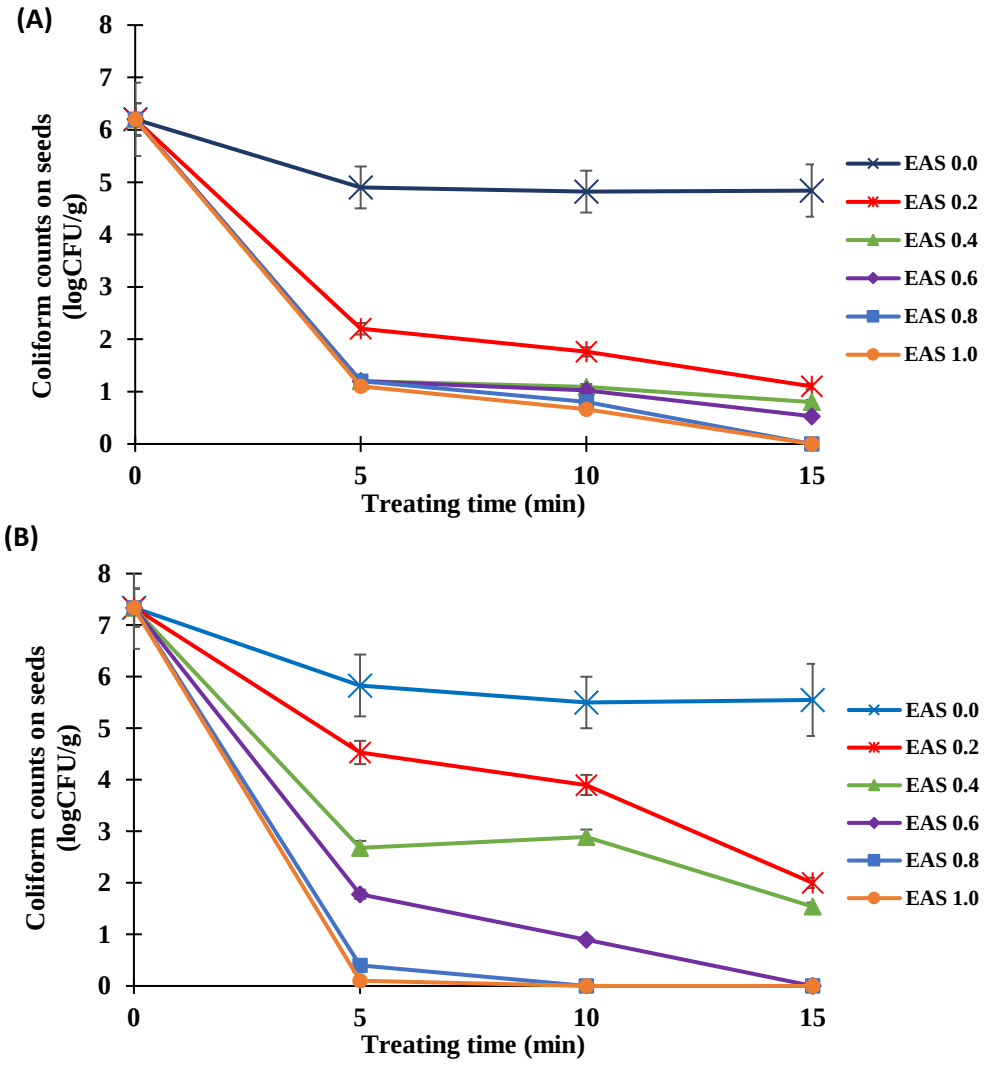

Figure 1. Effect of EAS on coliform counts on green mustard **(A)** and mung bean **(B)** seeds surface.

The effect of chlorine concentration in the EAS on coliform was more profound in the treatment of mung bean seed. The decrease in coliforms was linear with the increase in chlorine concentration at 5 min treatment; from 2.9 log CFU/g at ACC of 5.6 ppm to 7.33 log CFU/g (98.7% reduction) at ACC of 29.0 ppm. EAS treatment also showed to be more effective in mung beans compared to green mustard with no coliform detected after 10 min soaked in ≥ 23.1 ppm ACC solution, and 15 min soaked in ≥ 17.5 ppm ACC solution, respectively.

The disinfection activities of EAS against *E. carotovora* population occurred in almost the same trend for both types of seeds, as presented in Figure 2. The *E. carotovora* counts decrease almost linearly with the increase in chlorine concentration at all investigated times. The *E. carotovora* population on green mustard seeds before treatment reached as high as 5.97 log CFU/g. Seeds washed with water slightly reduced the bacteria on the seeds, about 1.01 - 1.47 log CFU/g, while seeds treated with EAS gave much better results with 100% reduction obtained in 5 min soaked in 29.0 ppm ACC concentration. The disinfection efficiency improved slightly more with a longer treatment time, and a 100% reduction was observed at ACC ≥ 23.1 ppm. Similarly, soaking mung bean seeds in EAS helped decrease *E. carotovora* counts significantly. EAS with ACC 29.0 ppm showed an extremely strong sterilisation effect with 100% efficacy in 5 min. At longer treatment, complete disinfection was achieved at ACC ≥ 23.1 ppm.

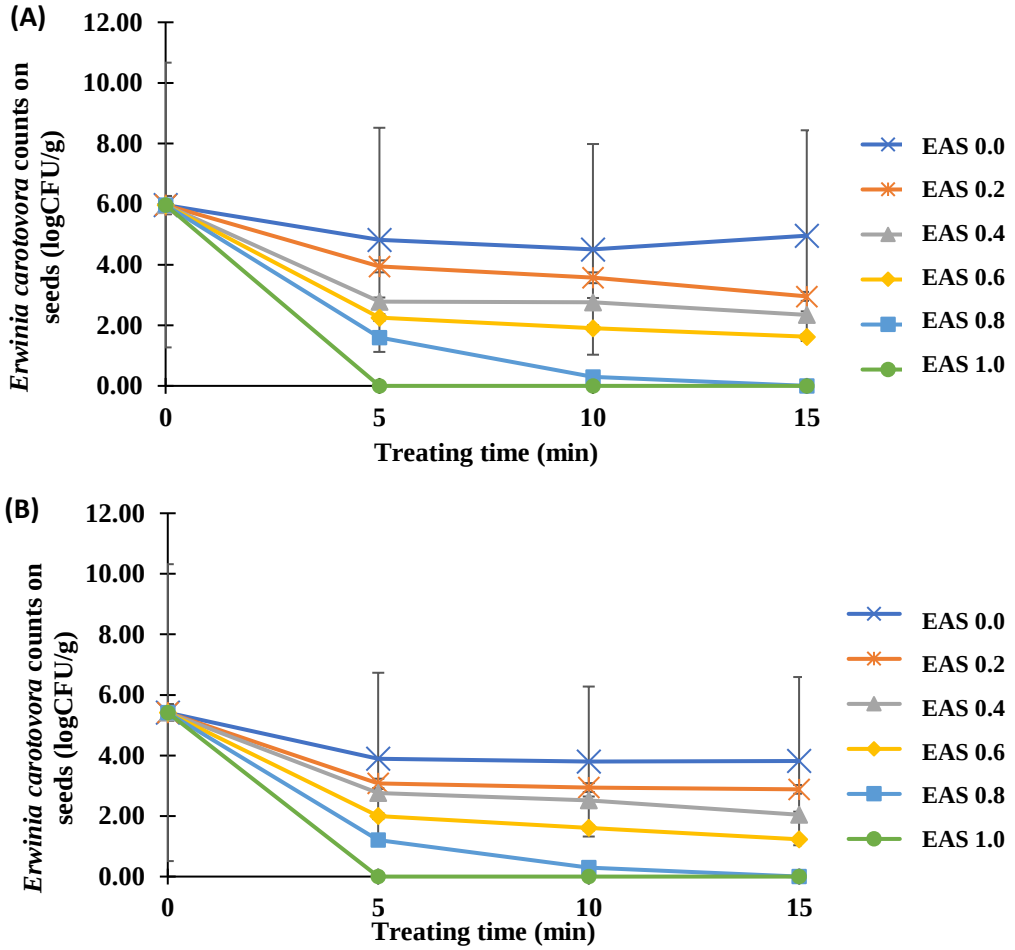

Figure 2. Effect of EAS on *Erwinia carotovora* population on green mustard **(A)** and mung bean **(B)** seed surface.

Coliform and Erwinia carotovora growth on treated seeds during storage

Coliform on untreated green mustard seeds (control sample) were initially 6.2 log CFU/g, and the counts later increased linearly with storage time, reaching 7.14 and 8.6 log CFU/g after 12 w stored at 8 and 28°C, respectively. In general, coliform counts increased after 12 w stored at both investigated temperatures; however, the increase was more rapid at 28°C. To investigate the effect of EAS treatment

on the development of microorganisms during storage, seeds were treated with different strengths of EAS (0, 0.2, 0.4, 0.6, 0.8, and 1 strength) for 5, 10, and 15 min before storage for 4, 8, and 12 w at 8 and 28°C. The results obtained are shown in Figure 3, where Figures 3A, 3B, and 3C show the variation in coliform counts on seeds after treatment and stored at 8°C, and Figures 3D, 3E, and 3F show those stored at 28°C. In these figures, the number of bacteria counted corresponds to colour code on scale bar. Variation in the area and position of a specific colour indicates a change in coliform count, implying a change in the level of sterilisation of seeds. Evidently, increasing the strength of the EAS and the treatment time resulted in greater antibacterial efficacy. This general trend remained almost unchanged with storage time at both investigated temperatures (8 and 28°C); however, the degree of sterilisation apparently decreased as the storage time and temperature increased. No coliform was detected in the seed samples treated for 15 min with EAS ≥ 0.8 strength and stored for 4 w at 8°C (Figure 3A). When seeds were treated with EAS ≥ 0.4 strength for ≥ 10 min, coliform counts were < 1 log CFU/g after 4 w at 8°C. The results indicated that the disinfection efficiency was maintained as the seeds were stored within 4 w at 8°C. When the storage time increased to 8 and 12 w, the degree of sterilisation significantly decreased, in which coliforms were found in all samples regardless of the concentration of ACC and the treatment time. This can be clearly observed on the 3-D surface plot shown in Figure 3. The purple area on the surface plot characterised by coliform count ≤ 1 log CFU/g decreased significantly as the storage time increased from 4 to 12 w. On the contrary, the red area coded for coliform counts ≥ 4.5 CFU/g increased in the same storage period. The decrease in sterilisation was found to be more rapid as treated seeds were stored at 28°C. As shown in Figure 3, coliforms were found in all EAS treated samples, and the purple area decreased rapidly when the storage time increased from 4 to 12 w. Only 3 EAS strength can generate coliform counts below 1 log CFU/g after 4 w. Seeds treated with EAS 0.6 and 0.8 strength for 15 min had 0.88 and 0.02 log CFU/g, and counts were 0.68 and 0.02 log CFU/g when seeds were treated with 1 strength solution for 10 and 15 min, respectively. For longer storage at 28°C, only samples treated with EAS of 0.8 and 1 strength can reach coliform < 1 log CFU/g.

Sterilisation effect against coliform on mung bean seeds was similar to that on green mustard seeds. No coliform was found in the seed samples treated for 15 min with EAS ≥ 0.8 strength, and ≥ 10 min with EAS 1 strength and stored for 4 w. As discussed earlier, higher sterilisation efficacy was observed on mung bean seed compared to green mustard seeds treated with EAS solution. This observation was unchanged as seeds were stored for different periods, regardless of the fact that initial coliform counts in mung bean seeds (7.8 ± 0.7 log CFU/g) were higher than the others (6.2 ± 0.7 CFU/g). Specifically, complete disinfection was only observed on green mustard seeds stored within 4 w at 8°C; however, this was achieved in mung bean seeds stored up to 8 w at 8°C and 4 w at 28°C. The bold purple area in the 3-D surface plots of coliform in EAS treated mung bean seeds corresponds to deep sterilisation (< 0.5 log CFU/g), and is larger than in EAS-treated green mustard seeds with the same storage time. The red region on the surface plot also increased along with increasing storage time and temperature. The observation showed that the storage of treated seeds at cold condition (8°C) resulted in better inhibitory effect than under normal temperature conditions (28°C). It has been postulated that cell function could be interfered with at low temperature, causing an inhibitory effect, and could be the reason for the lower bacterial count on seeds stored at 8°C (Nedwell, 1999).

Interestingly, an almost similar pattern for the coliform growth rate was found on both types of seeds at 8 and 28°C. The growth rate was rapid at low EAS strength (low ACC concentration) and higher storage temperature, and slow at high EAS strength and low storage temperature. This pattern was more profound on green mustard seeds with a clear first turn point at ~0.2 strength corresponding to a strong disinfection effect, and a second turn point at ~0.8 strength corresponding to a deep disinfection (< 0.5 log CFU/g). Meanwhile, the first turn points on the mung bean seeds occurred at > 0.2 strength, and were not clearly observed. Furthermore, the red surface area was larger in the mung bean seeds that extended from zero to 0.2 and 0.4 strength compared to 0.1 to 0.2 strength in the green mustard seeds. It only needed 0.2 strength (5.6 ppm ACC) to reduce 3.1 to 3.84 log CFU/g of coliform in green mustard seeds, while the reduction in mung bean seeds was only 1.8 to 3.1 log CFU/g. These results implied that the coliform

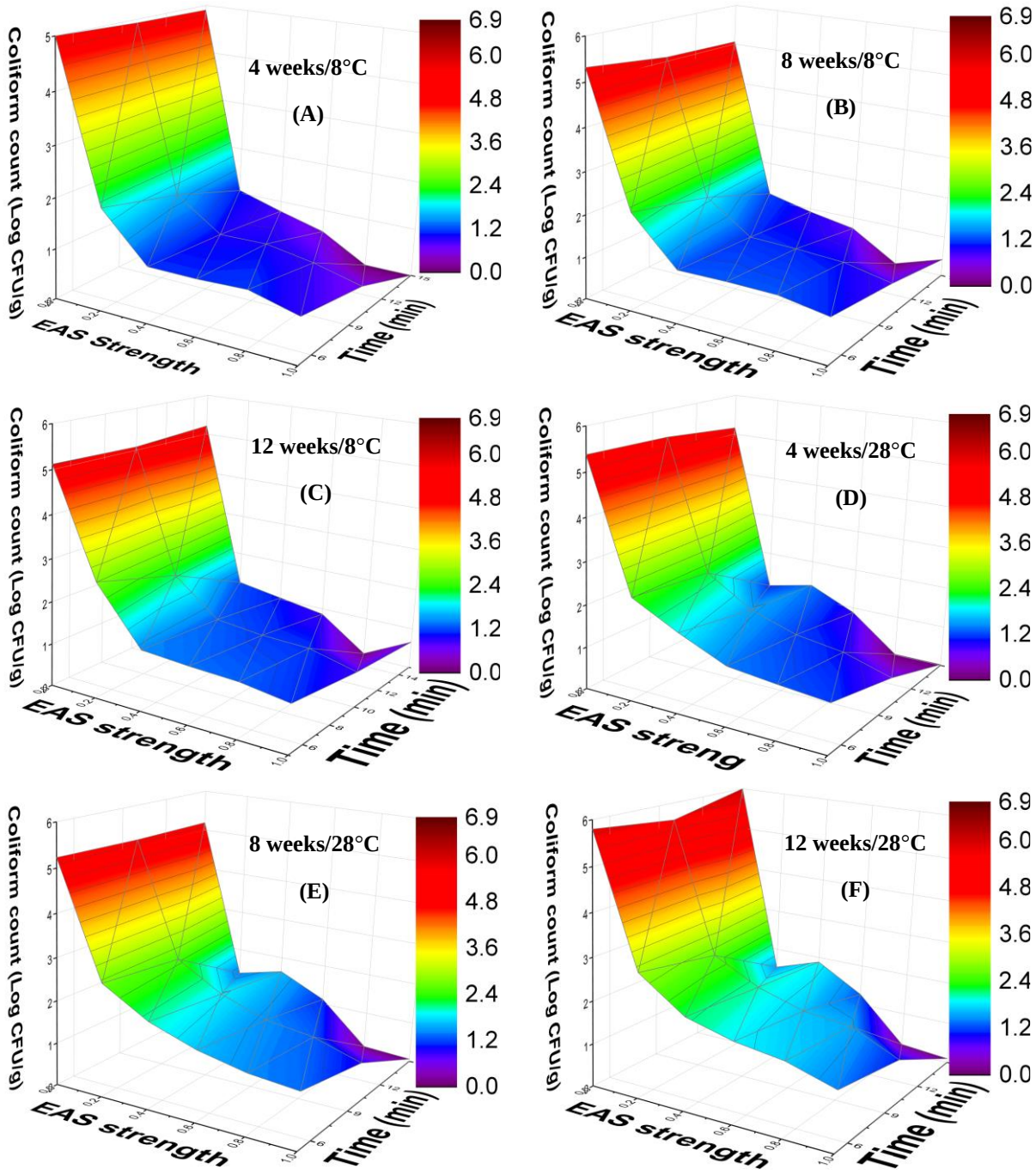

Figure 3. 3-D surface plot of coliform on EAS-treated green mustard seeds stored at 8°C for four weeks **(A)**, eight weeks **(B)**, and 12 weeks **(C)**; and at 28°C for four weeks **(D)**, eight weeks **(E)**, and 12 weeks **(F)**. EAS strength varied from 0 to 1, and seeds were treated for 5, 10, and 15 min.

population on green mustard seeds was more susceptible to EAS than that on mung bean seeds, and therefore, the disinfection was more effective on green mustard seeds. Since identical testing conditions were applied for both types of seeds, the higher disinfection effect was likely caused by differences in characteristics, particularly the different size of two seeds. Green mustards seeds had smaller sizes of approximately ~1 mm compared to mung bean seeds of ~2 - 3 mm, and therefore can hold EAS longer on the surface, and as a result, more bacteria were killed. However, regardless of the seeds tested, coliform counts were found in the range of 0.1 - 0.4 log CFU/g as the seeds were treated with EAS 1 strength for 15 min before being stored for 12 w at 28°C. Results indicated that the sterilisation of seeds

was achieved even after being stored for 12 w at room temperature.

To further evaluate the effect of EAS treatment on seed protection against the pathogen, the growth of *E. carotovora*, a root rotten disease caused by bacteria, during storage was investigated. In experiments with untreated seeds and those treated with water, the *E. carotovora* population on seeds stored at 8 and 28°C increased linearly with storage duration from 6.0 to 7.2 and 8.7 log CFU/g, respectively. This observation was in good agreement with that of coliform reported earlier. When seeds were treated with EAS solution, the inhibitory effect against *E. carotovora* was effectively achieved and maintained for weeks of storage. The relation between *E. carotovora* counted on seeds, EAS strength, and treatment time is shown in Figure 4. In general, the number of *E. carotovora* increased along with the decrease in the strength of EAS and the treatment time, and decreased with the longer storage time and the higher storage temperature. As seen in those figures, the red surface area (> 4.5 log CFU/g) increased, and the purple surface area decreased as storage time and temperature increased. No *E. carotovora was* detected on green mustard and mung bean seeds treated 15 min with EAS 0.8 strength stored for 4 w. With EAS 1 strength, the 5 min treatment seeds were free of *E. carotovora* for 4 w stored at 8°C. Mung bean seeds treated with EAS 1 strength for ≥ 10 min completely controlled *E. carotovora* after 12 w at 8°C and 4 w at 28°C. The results were much better for green mustard seed treated with EAS 1 strength for ≥ 10 min; no *E. carotovora* was detected after 12 w at 8°C and 8 w at 28°C, respectively. The present work confirmed that *E. carotovora* growth on green mustard and mung bean seeds was completely inhibited by treatment with EAS 1 strength (29 ppm ACC) for ≥ 10 min. These results suggested that the rotten disease in green mustard and mung bean seeds, which originates from *E. carotovora* contaminating the seeds could be prevented by treating seeds with EAS before storage and growth.

Effect of EAS treatment on seed germination and sprout growth

The seeds were treated with different EAS strengths, and in each EAS solution, the seeds were soaked for 5, 10, or 15 min. The samples were then stored for different periods at 8 or 28°C, respectively, to evaluate the effect of EAS treatment (EAS strength, treatment time, and storage temperatures) on seed germination and sprout growth. The results obtained are shown in Table 2. In general, the germination rate reached 97 to 100% for both seed species. The mung bean showed ≥ 98% rate, in which 77.44% of the samples achieved 100% germination. The germination rate of green mustard seeds was ≥ 97%, where only 16.54% of the samples reached 100% germination. However, for each seed species, no significant differences in the germination rate were observed between controls (samples treated with sterile water) and EAS-treated samples, with and without storage periods. These results indicated that the germination of green mustard and mung bean seeds was not negatively affected by the treatment with the tested EAS solution. Based on previous studies, the characteristics of the EAS solution, including pH and ACC concentration in the EAS solution, significantly influenced on the germination of seeds (Rui *et al*., 2011; Zhang *et al*., 2011; 2016). Zhang *et al*. (2016) indicated that the germination rate was considerably reduced as radish seeds were treated with an acidic or basic EAS solution, and the optimal germination was reached at pH ~6.5. In another study, they showed that the germination of mung bean decreased along with increasing ACC concentration; it decreased from a range of 90.7 - 92.0% to 79.7 - 86.0% as the ACC concentration increased from 20 to 40 ppm respectively (Zhang *et al*., 2011). In the present work, the EAS solution had pH values of 6.5 to 6.7, and ACC concentration ≤ 29.0 ppm, which were in the safe range, and did not affect seed germination. The effect of EAS treatment on seed germination observed in the present work, was therefore, in good congruence with that in previous works, confirming the effective usage of EAS for seed sterilisation (Rui *et al*., 2011; Zhang *et al*., 2011; 2016).

The lengths of green mustard and mung bean sprouts treated with EAS were around 98 - 107 and 64 - 70 mm, which were comparable to that of the control samples, from 99 - 106 and 65 - 68 mm, respectively (Table 2). This implied that EAS treatment did not negatively affect germination and sprout growth. Rui *et al*. (2011) indicated that the EAS solution with pH 2.16 only stunted the growth of mung bean sprouts, particularly in the later stage (≥ 96 h), and like the germination rate, optimal growth was achieved at pH 6.5. The present work

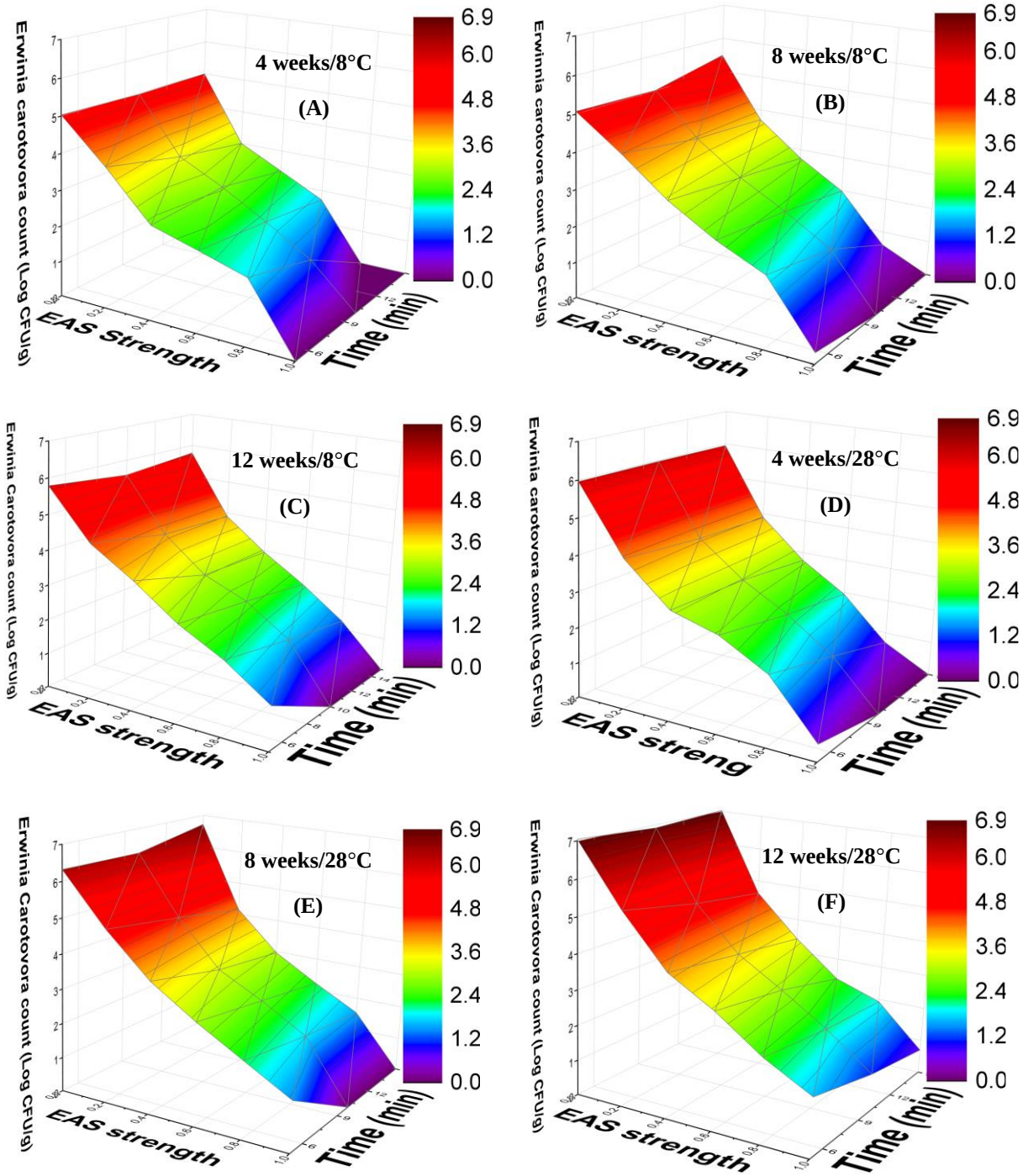

Figure 4. 3-D surface plot of *Erwinia carotovora* on treated green mustard seeds stored at 8°C for four weeks **(A)**, eight weeks **(B)**, and 12 weeks **(C)**; and at 28°C for four weeks **(D)**, eight weeks **(E)**, and 12 weeks **(F)**. EAS strength varied from 0 to 1, and seeds were treated for 5, 10, and 15 min.

Table 2. Germination rate and sprout length of mung bean seeds.

EAS strength	Treating time (min)	After treatment		Stored at 8°C						Stored at 28°C					
				After 4-week storage		After 8-week storage		After 12-week storage		After 4-week storage		After 8-week storage		After 12-week storage	
		Germination rate (%)	Sprout length (mm)	Germination rate (%)	Sprout length (mm)	Germination rate (%)	Sprout length (mm)	Germination rate (%)	Sprout length (mm)	Germination rate (%)	Sprout length (mm)	Germination rate (%)	Sprout length (mm)	Germination rate (%)	Sprout length (mm)
0	0	100 ± 0	66 ± 2	100 ± 0	66 ± 2	99 ± 1	66 ± 2	99 ± 1	65 ± 2	100 ± 0	66 ± 2	100 ± 0	66 ± 1	99 ± 1	65 ± 2
	5	100 ± 0	66 ± 2	99 ± 1	67 ± 1	99 ± 1	67 ± 2	99 ± 1	66 ± 2	100 ± 0	65 ± 2	99 ± 1	66 ± 3	100 ± 0	66 ± 2
	10	100 ± 0	65 ± 1	100 ± 0	66 ± 2	100 ± 0	67 ± 2	100 ± 0	67 ± 3	100 ± 0	66 ± 2	100 ± 0	66 ± 2	100 ± 0	66 ± 2
	15	100 ± 0	67 ± 1	100 ± 0	67 ± 2	100 ± 0	68 ± 1	100 ± 0	68 ± 3	100 ± 0	67 ± 2	100 ± 0	67 ± 1	100 ± 0	66 ± 3
0.2	5	100 ± 0	65 ± 2	100 ± 0	66 ± 2	99 ± 1	68 ± 3	100 ± 0	66 ± 3	99 ± 1	65 ± 3	100 ± 0	65 ± 3	100 ± 0	64 ± 3
	10	100 ± 0	69 ± 1	100 ± 0	68 ± 1	100 ± 0	69 ± 2	100 ± 0	67 ± 3	100 ± 0	66 ± 3	99 ± 1	67 ± 3	100 ± 0	66 ± 2
	15	100 ± 0	70 ± 1	100 ± 0	69 ± 2	100 ± 0	69 ± 3	100 ± 0	67 ± 2	100 ± 0	68 ± 2	100 ± 0	67 ± 3	99 ± 1	66 ± 2
0.4	5	100 ± 0	66 ± 2	98± 2	67 ± 2	100 ± 0	66 ± 2	100 ± 0	65 ± 1	99 ± 1	67 ± 2	100 ± 0	66 ± 3	100 ± 0	66 ± 3
	10	99± 1	68 ± 1	100 ± 0	68 ± 2	100 ± 0	67 ± 2	98 ± 2	67 ± 2	100 ± 0	69 ± 3	100 ± 0	67 ± 2	100 ± 0	67 ± 2
	15	100 ± 0	68 ± 1	100 ± 0	69 ± 1	100 ± 0	67 ± 2	100 ± 0	67 ± 3	100 ± 0	69 ± 3	99 ± 1	67 ± 3	100 ± 0	67 ± 1
0.6	5	100 ± 0	69 ± 2	100 ± 0	68 ± 2	99 ± 1	68 ± 2	100 ± 0	68 ± 3	100 ± 0	67 ± 2	99 ± 1	68 ± 3	100 ± 0	67 ± 3
	10	98 ± 2	68 ± 2	100 ± 0	69 ± 2	100 ± 0	68 ± 3	100 ± 0	68 ± 3	100 ± 0	67 ± 3	100 ± 0	68 ± 3	99 ± 1	67 ± 2
	15	100 ± 0	69 ± 1	100 ± 0	69 ± 3	100 ± 0	69 ± 2	100 ± 0	68 ± 2	100 ± 0	68 ± 3	100 ± 0	67 ± 3	99 ± 1	67 ± 1
0.8	5	100 ± 0	67 ± 1	99 ± 1	67 ± 2	100 ± 0	68 ± 2	100 ± 0	66 ± 2	99 ± 1	67 ± 3	100 ± 0	67 ± 2	100 ± 0	66 ± 2
	10	100 ± 0	67 ± 1	100 ± 0	68 ± 2	100 ± 0	68 ± 3	100 ± 0	68 ± 2	100 ± 0	67 ± 2	100 ± 0	67 ± 3	100 ± 0	66 ± 2
	15	100 ± 0	68 ± 2	100 ± 0	69 ± 2	100 ± 0	67 ± 3	99 ± 1	68 ± 3	100 ± 0	67 ± 2	100 ± 0	67 ± 3	100 ± 0	67 ± 2
1.0	5	98 ± 2	69 ± 1	100 ± 0	67 ± 3	99 ± 1	68 ± 2	99 ± 1	67 ± 2	99 ± 1	68 ± 2	99 ± 1	68 ± 3	100 ± 0	67 ± 3
	10	100 ± 0	68 ± 2	100 ± 0	68 ± 2	100 ± 0	69 ± 2	100 ± 0	68 ± 3	100 ± 0	69 ± 2	100 ± 0	68 ± 1	100 ± 0	69 ± 1
	15	100 ± 0	68 ± 1	100 ± 0	69 ± 2	100 ± 0	68 ± 2	100 ± 0	68 ± 2	100 ± 0	69 ± 3	100 ± 0	68 ± 1	99 ± 1	68 ± 2

used EAS having ACC concentration ≤ 29.0 ppm and pH ~6.5, and as a result, the sprout growth was not affected. These results further confirmed that treatment of mung bean and green mustard seeds with an EAS solution having pH ~6.5 and ACC concentration 5.6 - 29.0 ppm helped to effectively control the bacterial population in seeds, and did not affect seed germination and sprout growth. This sterilisation method could therefore be suitable not only for food processing, but also for agriculture applications to prevent potential disease pathogen transfer.

Conclusion

The EAS produced from diluted KCl concentrate (1 g/L) showed great disinfection efficacy against coliform and *E. carotovora* population in mung bean and green mustard seeds. The sterilisation efficacy of EAS was significantly improved when the available chlorine concentration and/or treatment time was increased. The storage of treated seeds under cold conditions (8°C) resulted in a better inhibitory effect than the normal temperature condition (28°C). Complete seed sterilisation was achieved by treatment with an EAS solution, having chlorine concentrations available of 29.0 ppm, and stored in 12 w at 8°C, or 4 to 8 w at 28°C. Interestingly, when treating seeds, this EAS solution did not affect germination and sprout growth. These indicated that seeds can be used right after the treatment with EAS or stored for 12 w at 8°C, or 4 to 8 w at 28°C without worrying about microbial contamination. The present work proposed an efficient and inexpensive method of sterilising and controlling pathogens that originate from seeds before growing sprouts, thus improving the hygiene of sprout salad products.

Acknowledgement

The present work was financially supported by the Institute of Environmental Technology, Vietnam Academy of Science and Technology.

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