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# Large-Scale Gaseous Acetic Acid Treatment to Disinfect Alfalfa Seeds Inoculated with *Escherichia coli*

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**Abstract**

Most outbreaks of foodborne illness related to sprout consumption are ascribed to bacterial contamination of its seeds, and they need disinfection before sprouting. Recently, gaseous acetic acid (GAA) treatment received great attention as a method for seed disinfection. In this study, the effect of GAA treatment on alfalfa seed disinfection was evaluated in a large-scale device to simulate practical applications. Alfalfa seeds (3 kg) inoculated with *Escherichia coli* were treated with 8.7% (vol/vol) GAA at 55°C for 1–3 h. The population of *E. coli* was significantly reduced ($p < 0.05$), and the reduction was larger with longer exposure times. After 3-h treatment, a maximum decrease by more than 5 log colony-forming units/g was observed. The germination ratio of alfalfa seeds was not affected by the treatments under all the conditions. The results indicated that the GAA treatment has a potential for practical application to reduce the risk of foodborne illness caused by consumption of sprouts.

## Introduction

Many outbreaks related to sprouts have been reported, and at least five outbreaks are listed in the United States from 2009 to 2012 (CDC, 2013a, b). Sprout seeds are contaminated with pathogenic bacteria via various media such as soil, fertilizer, and water. Therefore, seed disinfection before germination is important to reduce the risk of foodborne illness.

Aqueous chlorine treatment is widely used to disinfect agricultural products. However, the disinfection ability of chlorine treatments has been limited (Montville and Schaffner, 2004). A previous report demonstrated that gaseous acetic acid (GAA) treatment is effective as an alternative method to chlorine treatment, and a more than 5 log colony-forming units (CFU)/g reduction of pathogenic bacteria was achieved in a laboratory-scale test (Nei *et al*., 2011). Although the treatment is not officially recommended by national or international authorities, GAA treatment would be attractive to disinfect the seeds. However, since the amount of seeds often affects the efficacy of seed disinfection (Fransisca *et al*., 2012), the results in the laboratory-scale test are not always applicable to industrial processing. Therefore, this study aimed at examining the feasibility of an industrial large-scale GAA treatment in decontaminating alfalfa seeds inoculated with *Escherichia coli*.

## Materials and Methods

### *Test strains and procedure for inoculation*

Three isolated strains of nonpathogenic *E. coli* (080611-3 from spinach, 080514-2 from bean sprout, and 080618-8 from celery) were used due to biosafety concerns in the commercial production facility where this study was conducted. Each strain was cultured at 37°C in tryptic soy broth (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) for 24 h. Cells from each strain were collected by centrifugation ($3000 \times g$ for 10 min at 4°C) and resuspended in 40 mL of sterile phosphate-buffered saline (pH 7.2) solution. The centrifugation and resuspension was repeated twice. Equal volumes of each cell suspension were mixed to obtain a bacterial cocktail of 9.0 log CFU/mL. Alfalfa seeds (3 kg) produced in the United States were soaked into 6 L of the bacterial cocktail and agitated for 5 min. After the inoculum was decanted, the seeds were dried on a clean bench at room temperature for 8 h. The inoculum level of the seeds was 7.0 log CFU/g as a total number of three *E. coli* strains.

### *GAA treatment*

A large-scale gas fumigation chamber (AG1000-AS, Daisey Machinery Co. Ltd., Saitama, Japan) was used. The

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chamber (860 mm × 833 mm × 943 mm) is equipped with a heating device and a rotating drum (1.5 rpm). Under each experimental condition, 3 kg of alfalfa seeds were placed in the rotating drum inside the chamber. Acetic acid (Wako Pure Chemical Co. Ltd., Tokyo, Japan) was heated for 10 min until vaporization, and the resulting GAA was introduced into the chamber. According to previous research (Nei *et al*., 2011), the GAA concentration and temperature were kept at 8.7% (vol/vol) and 55°C, respectively. The treatment was carried out for 1–3 h at the given gas and temperature conditions. After completion of the treatment, GAA was exhausted to collect the samples.

### *Microbial analysis*

Twenty-five grams of seeds were randomly sampled 10 times from 3 kg of control and treated seeds. The seeds were placed in a stomacher bag with 225 mL of peptone-buffered water (pH 7.2) and pummeled for 60 s. The pour plate was selected to count the *E. coli* population because a lower detection limit was needed. The sample suspensions were appropriately diluted with peptone-buffered water and pour-plated in quadruplicate on Chromocult coliform agar (Merck, Darmstadt, Germany).

### *Germination ratio and sprout growth*

Approximately 300 seeds were counted and placed on a paper towel in a Petri dish, and a proper amount of water was applied to maintain a humidity condition. The seeds were stored at 25°C for 4 days, and the germination ratio (percent) was calculated from the number of germinated seeds.

### *Statistical analysis*

Two independent experiments were performed and data were expressed as the mean value ± standard error. Significant differences in mean values were judged by the Tukey–Kramer multiple comparison method at a 5% level of significance using SPSS (SPSS Inc., Chicago, IL).

## Results and Discussion

The results of the GAA treatment on *E. coli* counts on alfalfa seeds are summarized in Table 1. Approximately 7.0 log CFU/g of *E. coli* were recovered from the inoculated seeds. The GAA treatment for 1 h significantly reduced *E. coli* counts by 2.6 log CFU/g ($p < 0.05$). A larger reduction in the *E. coli* population was achieved with longer exposure times and after 2- and 3 h-treatments, the population was reduced by 4.8 and 5.1 log CFU/g, respectively. In a laboratory scale, more than 5 log CFU/g reduction of *E. coli* O157:H7 on alfalfa seeds was achieved by a GAA treatment for 2 h (Nei *et al*., 2011). Although pathogenic bacteria were not used in this study, the large reductions in *E. coli* counts indicate that the GAA treatment may be applicable on a large industrial scale. The GAA treatment does not need water for disinfection, and it has a great potential to save water resources; however, it should be noted that a completely sealed device is required due to the toxicity that the high concentration of acetic acid used may have on the facility workers (CDC, 2010).

Germination ratios of alfalfa seeds after the GAA treatments are also shown in Table 1. No significant difference in the germination ratio was observed under each condition between control and treated seeds, which is in agreement with other reports (Weissinger *et al*., 2001). Although GAA treatment may be applicable to industrial processing, further studies on optimization of the treatment conditions will be necessary to minimize the residual smell of acetic acid on the products for its practical application. In addition, further experiments are needed to investigate the mode of action of GAA on microorganisms and the quality changes produced by GAA treatments on sprouts, such as shelf-life and color.

Table 1. Effect of Gaseous Acetic Acid Treatment on a Population of *Escherichia coli* Inoculated on Alfalfa Seeds and on the Germination Ratios of Alfalfa Seeds

| *Treatment conditions* | *Population of* E. coli[a] *(log CFU/g)* | *Germination ratio 1*[b] *(%)* | *Germination ratio 2*[c] *(%)* |
|---|---|---|---|
| Control | 7.0 ± 0.1[a] | 82 | 79 |
| Gaseous acetic acid (8.7% at 55°C) | | | |
| 60 min | 4.4 ± 0.2[b] | 84 | 81 |
| 120 min | 2.1 ± 0.2[c] | 84 | 81 |
| 180 min | 1.9 ± 0.2[c] | 83 | 80 |

[a]The population data are represented by the mean of two independent trials with 10 samples and the standard error ($n = 20$). Within individual columns, the values followed by different letters are significantly different ($p < 0.05$).
[b]Germination ratios, including the seeds that did not grow healthily.
[c]Germination ratios, excluding the seeds that did not grow healthily.

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## Disclosure Statement

No competing financial interests exist.

## References

[CDC] Centers for Disease Control and Prevention. Reports of selected *E. coli* outbreak investigations. 2013a. Available at: http://www.cdc.gov/ecoli/outbreaks.html, accessed September 1, 2013.

CDC. Reports of selected *Salmonella* outbreak investigations. 2013b. Available at: http://www.cdc.gov/salmonella/outbreaks.html, accessed September 1, 2013.

CDC. International chemical safety cards. Acetic acid. 2010. Available at: http://www.cdc.gov/niosh/ipcsneng/neng0363.html, accessed October 29, 2013.

Fransisca L, Park HK, Feng H. *E. coli* O157:H7 population reduction from alfalfa seeds with malic acid and thiamine dilauryl sulfate and quality evaluation of the resulting sprouts. J Food Sci 2012;77:M121–M126.

Montville R, Schaffner DW. Analysis of published sprout seed sanitization studies shows treatments are highly variable. J Food Prot 2004;67:758–765.

Nei D, Bari ML, Enomoto K, Inatsu Y, Kawamoto S. Disinfection of radish and alfalfa seeds inoculated with *Escherichia coli* O157:H7 and *Salmonella* by a gaseous acetic acid treatment. Foodborne Pathog Dis 2011;8:1089–1094.

Weissinger WR, McWatters KH, Beuchat LR. Evaluation of volatile chemical treatments for lethality to *Salmonella* on alfalfa seeds and sprouts. J Food Prot 2001;64:442–450.

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