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Effect of aerosolized malic acid against *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Escherichia coli* O157:H7 on spinach and lettuce

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ABSTRACT

The purpose of this study is to investigate the efficacy of aerosolized malic acid for inhibiting foodborne pathogens (*Listeria monocytogenes*, *Salmonella* Typhimurium, and *Escherichia coli* O157:H7) on spinach and lettuce. Spinach and lettuce were inoculated with a cocktail containing three strains of each pathogen then treated with aerosolized malic acid at the concentration of 0.25%, 0.5%, 1% and 2% for 10, 30, 50, and 100 min at room temperature (22 ± 2 °C). The control showed that the levels of three pathogens did not significantly when treated for 50 min or less. However, the levels of three pathogens were significantly reduced by treatment with aerosolized malic acid. In particular, aerosolized 2% malic acid for 100 min was the most effective treatment to reduce the three pathogens on spinach and lettuce. The reduction levels of *L. monocytogenes*, *S.* Typhimurium, and *E. coli* O157:H7 on spinach and lettuce were 3.35, 4.10, 3.67, and 3.85, 5.02, 3.35 $\log_{10}$ CFU/g, respectively. Aerosolized malic acid was shown to be effective at killing foodborne pathogens on spinach and lettuce without deteriorating the quality. Therefore, aerosolized malic acid might be used as an alternative sanitizer to increase the microbial safety of fresh produce during transportation and storage.

1. Introduction

Fresh produce, especially fruits and vegetables, is an essential part of the diet of people around the world, and its consumption has increased in recent years (Park, Alexander, Tarlor, Costa, & Kang, 2008). As a result of this increase in consumption, an increase in the frequency of outbreaks of illness associated with raw or minimally processed fruits and vegetables has occurred (Lee, Yun, Fellman, & Kang, 2002). Pathogenic bacterial contamination originates from irrigation or wash water, animal or municipal sewage-based fertilizers, infected workers, and food processing facilities with poor sanitation (Lee, Costello, & Kang, 2004). Pathogenic microorganisms of most concern in minimally processed fresh products include *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* (Lee et al., 2004). These foodborne pathogens have been implicated in outbreaks of illness linked to the consumption of contaminated fresh produce such as spinach and lettuce (Lee et al., 2004). For instance, *E. coli* O157:H7 infections were reported in 199 persons in 26 states in association with consumption of fresh spinach-containing products between August 9 and September 5, 2006 (Centers for Disease Control and Prevention, 2006). In December 2006, lettuce contaminated with *E. coli* O157:H7 led to 71 infections in 5 states that included 53 hospitalizations and 8 cases of hemolytic uremic syndrome (HUS) (FDA, 2006). Therefore, treatment with sanitizers is a very important step for preventing these incidents of foodborne outbreaks.

Organic acids have a long history of being utilized as food additives, and preservatives for preventing food deterioration and extending the shelf life of perishable food ingredients (Ricke, 2003) Organic acids are generally recognized as safe (GRAS) (Izat, Colberg, Adams, Reiber, & Waldroup, 1989) and can be used as sanitizers of fresh produce because of their bactericidal activity. Several researchers have been studying the effects of organic acids for disinfecting fresh produce. For examples, Akbas and Ölmez (2007) studied the antimicrobial activity of lactic acid, citric acid, acetic acid, and ascorbic acid against *E. coli* ATCC 25922 and *L. monocytogenes* ATCC 7644 on iceberg lettuce. Massilia, Melgar, and Belloso (2009) reported inactivation of *E. coli* O157:H7, *Salmonella* Typhimurium, and *L. monocytogenes* in apple, pear and melon juices following treatment with malic acid. Venkitanarayanan, Lin, Bailey, and Doyle (2002) reported that lactic acid with H_2O_2 almost

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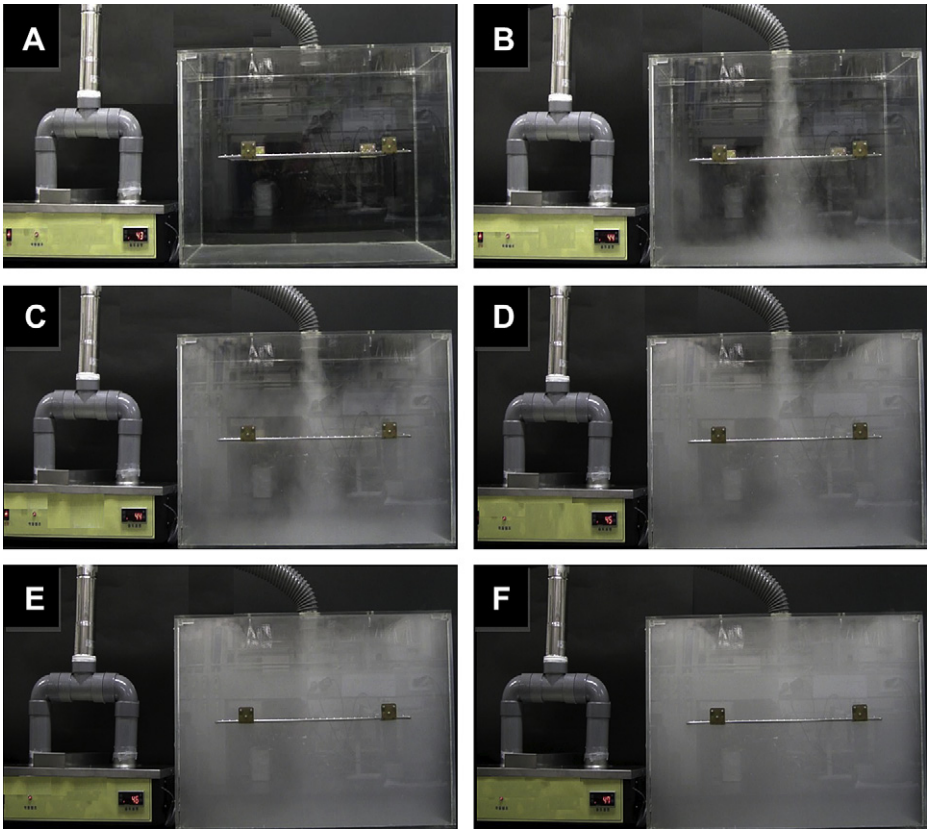

Fig. 1. Digital picture of aerosolized 2% malic acid in a model cabinet system: (A) 0 sec, (B) 4 sec, (C) 8 sec, (D) 12 sec, (E) 16 sec, and (F) 20 sec.

completely inactivated *E. coli* O157:H7, *Salmonella* Enteritidis, and *L. monocytogenes*.

For sanitizing food contact surfaces, sanitizers can be used in aqueous and gaseous forms (Lee et al., 2004). Aqueous sanitizers may fail to adequately contact injured or inaccessible surfaces, and thus not kill foodborne pathogens (Lee et al., 2004). Also, gaseous sanitizers are limited because of the sophisticated apparatus needed for gas generation and the scarcity of applicable sanitizers. However, aerosolization circumvents these limitations inherent with aqueous and gaseous sanitizers. According to Oh, Dancer, and Kang (2005), aerosolization is the dispersion of liquid as a fine mist in air and has clinical applications in respiratory medical treatments. Also, they observed that treatment with an aerosolized sanitizer results in better control than use of the aqueous form for reduction of foodborne pathogens (*L. monocytogenes*, *S.* Typhimurium, and *E. coli* O157:H7) on lettuce. This system has the added advantage that the mist form of sanitation can be used during transportation and storage. Therefore aerosolization can be used as a novel method for applying aqueous sanitizers in order to improve their efficacy. Foodborne pathogens present on fresh produce can grow during transportation and storage. Thus, it is necessary to find an effective method to inactivate foodborne pathogens on fresh produce during these extended intervals. Aerosolization might be used as an alternative sanitizing method during these periods. Therefore, this study was conducted to evaluate the efficacy of aerosolized malic acid for inhibiting *L. monocytogenes*, *S.* Typhimurium, and *E. coli* O157:H7 on spinach and lettuce.

2. Materials and methods

2.1. Cultures and cell suspension

Three strains each of *L. monocytogenes* (ATCC 7644, ATCC 19114 and ATCC 19115), *S.* Typhimurium (ATCC 19585, ATCC 43971, and DT 104), and *E. coli* O157:H7 (ATCC 35150, ATCC 43889 and ATCC 43890) were obtained from the School of Food Science Culture Collection at

Table 1
Populations (Log_{10} CFU/g) of *Listeria monocytogenes* inoculated on spinach exposed to aerosolized distilled water (control) and malic acid [0.25, 0.5, 1, 2% (w/v)] for 10, 30, 50, and 100 min at room temperature (22 ± 2 °C).

Time (min)	Treatments				
	Water (control)	0.25% Malic acid	0.5% Malic acid	1% Malic acid	2% Malic acid
0	6.97 ± 0.38^{a}	6.97 ± 0.38^{a}	6.97 ± 0.38^{a}	6.97 ± 0.38^{a}	6.97 ± 0.38^{a}
10	6.89 ± 0.38^{ax}	6.66 ± 0.05^{axy}	6.63 ± 0.13^{abxy}	6.29 ± 0.11^{aby}	6.25 ± 0.43^{aby}
30	6.81 ± 0.36^{ax}	6.51 ± 0.11^{abxy}	6.29 ± 0.12^{abyz}	5.96 ± 0.04^{bcz}	5.26 ± 0.24^{bcdZ}
50	6.60 ± 0.30^{ax}	6.36 ± 0.31^{abx}	5.98 ± 0.10^{bxy}	5.29 ± 0.53^{cy}	4.43 ± 0.67^{cdz}
100	6.37 ± 0.30^{ax}	5.70 ± 0.70^{bxy}	4.75 ± 0.77^{cyz}	4.35 ± 0.62^{dz}	3.62 ± 0.87^{dz}

[a–d]Means with the same letter within a column are not significantly different ($P < 0.05$).
[x–z]Means with the same letter within a row are not significantly different ($P < 0.05$).

Table 2
Populations (Log_{10} CFU/g) of *Salmonella* Typhimurium inoculated on spinach exposed to aerosolized distilled water (control) and malic acid [0.25, 0.5, 1, 2% (w/v)] for 10, 30, 50, and 100 min at room temperature (22 ± 2 °C).

Time (min)	Treatments				
	Water (control)	0.25% Malic acid	0.5% Malic acid	1% Malic acid	2% Malic acid
0	7.42 ± 0.05^{a}	7.42 ± 0.05^{a}	7.42 ± 0.05^{a}	7.42 ± 0.05^{a}	7.42 ± 0.05^{a}
10	7.22 ± 0.28^{ax}	7.01 ± 0.30^{abx}	6.95 ± 0.31^{ax}	6.80 ± 0.17^{ax}	6.76 ± 0.08^{ax}
30	7.12 ± 0.26^{ax}	6.77 ± 0.23^{bx}	6.78 ± 0.11^{abx}	6.00 ± 0.30^{by}	5.69 ± 0.13^{by}
50	6.94 ± 0.26^{abx}	6.57 ± 0.05^{bxy}	6.07 ± 0.32^{byz}	5.57 ± 0.19^{bz}	5.09 ± 0.68^{bZ}
100	6.61 ± 0.38^{bx}	5.88 ± 0.16^{cx}	4.61 ± 0.81^{cy}	4.37 ± 0.75^{cyz}	3.32 ± 0.59^{cz}

$^{a-c}$Means with the same letter within a column are not significantly different ($P < 0.05$).
$^{x-z}$Means with the same letter within a row are not significantly different ($P < 0.05$).

Washington State University (Pullman, WA, USA) and then used to inoculate onto spinach or lettuce. Each strain of *L. monocytogenes*, *S.* Typhimurium, and *E. coli* O157:H7 was cultured in 5 ml Tryptic Soy Broth (TSB: Difco, Becton Dickinson, Sparks, MD, US) at 37 °C for 24 h, harvested by centrifugation at 4000×*g* for 20 min at 4 °C and washed three times with buffered peptone water (pH 7.0, Difco). The final cell concentration in the buffer was approximately 8–9 log_{10} CFU/ml. After that, the three strains of each foodborne pathogen were combined with those of the other pathogens to construct a nine-strain mixed culture cocktail. This multi-pathogen culture cocktail was used for subsequent experimentation.

2.2. Sample preparation and inoculation

Commercial spinach and lettuce were purchased from a local market (Seoul, Korea) on the day before the experiment. Spinach and lettuce leaves (15 g) were separated and placed on sterile aluminum foil in a biosafety hood. Then 100 μl of the pathogen cocktail was inoculated onto the surface of spinach and lettuce leaves by depositing droplets at 15–20 locations with a micro-pipettor. Inoculated leaves were air-dried for 2 h in the hood with the fan running at room temperature (22 ± 2 °C).

2.3. Preparation of treatments

Aqueous solutions containing 0.25%, 0.5%, 1% and 2% (w/v) malic acid (Malic acid, 99%, Samchunpure Chemical Co. Ltd., Pyeongtaek, Korea) were prepared using sterile distilled water. Freshly prepared solutions were used within 30 min at room temperature (22°C ± 2 °C). Distilled water served as a control.

2.4. Antimicrobial aerosol treatment

A model glass cabinet (80 × 50 × 50 cm) was used in this study. The cabinet was sealed and aerosolized mist was routed from a new built nebulizer (DRWL-2000, Doore Industrial Co., Gyeonggi, Korea). The tube entered the lid of the cabinet. Inoculated spinach and lettuce leaves were treated with aerosolized malic acid for 10, 30, 50, and 100 min in the cabinet. The nebulizer left switched on for up to 10, 30, 50, and 100 min. Aqueous malic acid was atomized into approximately 5.42–11.42 μm particles. All tests were performed at room temperature (22 ± 2 °C).

2.5. Bacterial enumeration

After 10, 30, 50 and 100 min of treatment, spinach and lettuce leaves were placed in stomacher bags containing 50 ml D/E neutralizing broth (Difco) and homogenized for 2 min using a stomacher (EASYMIX, AES Chemuex, Rennes, France). After homogenization, samples were serially 10-fold diluted with 9 ml sterile buffered peptone water and 0.1 ml of sample or diluent was plated onto each selective agar. Oxford Agar Base (OAB; Difco) with antimicrobic supplement (Bacto Oxford Antimicrobic Supplement, Difco), Xylose Lysine Desoxycholate Agar (XLD; Difco), and Sorbitol MacConkey agar (SMAC; Difco) were used as selective media to enumerate *L. monocytogenes*, *S.* Typhimurium, and *E. coli* O157:H7, respectively. All plates were incubated at 37 °C for 24–48 h, then typical colonies characteristic of *L. monocytogenes*, *S.* Typhimurium and *E. coli* O157:H7 were enumerated.

2.6. Color measurement

Color change of spinach and lettuce was measured at 3 locations on each leaf at 0, 1, 3, 5, and 7 days after treatment using a Minolta colorimeter (model CR300, Minolta Co., Osaka, Japan). Colors were expressed as lightness (L^* values), redness-greenness (a^* values) and blueness-yellowness (b^* values) of spinach and lettuce samples.

2.7. Statistical analysis

All experiments were repeated three times with duplicate samples and averages of duplicate plate counts from three replications were converted to units of log_{10} CFU/g. Data were analyzed by the ANOVA procedure of SAS (Version 8.1. SAS Institute Inc., Cary, NC, USA) for a completely randomized design. When the main

Table 3
Populations (Log_{10} CFU/g) of *Escherichia coli* O157:H7 inoculated on spinach exposed to aerosolized distilled water (control) and malic acid [0.25, 0.5, 1, 2% (w/v)] for 10, 30, 50, and 100 min at room temperature (22 ± 2 °C).

Time (min)	Treatments				
	Water (control)	0.25% Malic acid	0.5% Malic acid	1% Malic acid	2% Malic acid
0	7.77 ± 0.20^{a}	7.77 ± 0.20^{a}	7.77 ± 0.20^{a}	7.77 ± 0.20^{a}	7.77 ± 0.20^{a}
10	7.73 ± 0.22^{ax}	7.66 ± 0.21^{ax}	7.24 ± 0.51^{axy}	6.90 ± 0.35^{abxy}	6.54 ± 0.69^{bz}
30	7.52 ± 0.36^{abx}	7.15 ± 0.45^{abxy}	6.78 ± 0.96^{abxyz}	6.16 ± 0.56^{bcyz}	5.70 ± 0.39^{bcz}
50	7.36 ± 0.26^{abx}	6.87 ± 0.44^{bxy}	6.17 ± 1.29^{abxyz}	5.29 ± 1.05^{cdyz}	4.79 ± 0.85^{cdz}
100	6.98 ± 0.34^{bx}	6.17 ± 0.32^{bx}	5.39 ± 0.69^{by}	4.59 ± 0.67^{dyz}	4.10 ± 0.33^{dz}

$^{a-d}$Means with the same letter within a column are not significantly different ($P < 0.05$).
$^{x-z}$Means with the same letter within a row are not significantly different ($P < 0.05$).

Table 4
Populations (Log_{10} CFU/g) of *Listeria monocytogenes* inoculated on lettuce exposed to aerosolized distilled water (control) and malic acid [0.25, 0.5, 1, 2% (w/v)] for 10, 30, 50, and 100 min at room temperature (22 ± 2 °C).

Time (min)	Treatments				
	Water (control)	0.25% Malic acid	0.5% Malic acid	1% Malic acid	2% Malic acid
0	7.35 ± 0.33^{a}	7.35 ± 0.33^{a}	7.35 ± 0.33^{a}	7.35 ± 0.33^{a}	7.35 ± 0.33^{a}
10	7.26 ± 0.07^{ax}	6.99 ± 0.26^{abxy}	6.89 ± 0.31^{abxy}	6.89 ± 0.19^{abxy}	6.52 ± 0.44^{aby}
30	7.12 ± 0.38^{ax}	6.91 ± 0.31^{abx}	6.82 ± 0.31^{abx}	6.14 ± 0.38^{by}	5.18 ± 0.42^{bcz}
50	6.93 ± 0.24^{ax}	6.81 ± 0.22^{abx}	6.76 ± 0.33^{abx}	5.31 ± 0.54^{cy}	4.19 ± 0.50^{cdz}
100	6.83 ± 0.23^{ax}	6.71 ± 0.27^{bx}	6.34 ± 0.27^{bx}	4.77 ± 0.72^{cy}	3.50 ± 0.17^{dz}

[a–d]Means with the same letter within a column are not significantly different ($P < 0.05$).
[x–z]Means with the same letter within a row are not significantly different ($P < 0.05$).

effect was significant ($P < 0.05$), means were separated using the Duncan's multiple range test.

3. Results and discussion

Fig. 1 shows diffusion of aerosolized 2% malic acid in the model cabinet with nebulizer. After activating the nebulizer, aerosolized 2% malic acid dispersed evenly in the cabinet. The cabinet rapidly filled with aerosolized 2% malic acid, and was visually saturated after 20 s.

The populations of *L. monocytogenes*, *S.* Typhimurium and *E. coli* O157:H7 before and after treatment with aerosolized distilled water and malic acid are shown in Tables 1–3. The initial population of *L. monocytogenes* on spinach was 6.97 log_{10} CFU/g, and was not significantly ($P \geq 0.05$) reduced by treatment with aerosolized distilled water for 100 min (Table 1). Whereas, when inoculated spinach was treated with aerosolized malic acids (0.25–2%), levels of *L. monocytogenes* were decreased significantly ($P < 0.05$). In particular, aerosolized 2% malic acid for 100 min was the most effective treatment in terms of reducing *L. monocytogenes*, and resulted in a higher reduction (3.35 log_{10} CFU/g reduction) compared to other treatments.

Spinach leaves were inoculated with 7.42 log_{10} CFU/g of *S.* Typhimurium (Table 2). *S.* Typhimurium was significantly affected by the control treatment (distilled water) after 100 min ($P < 0.05$), although reduction levels were less than 1 log_{10} CFU/g. When spinach leaves were treated with aerosolized malic acid, levels of *S.* Typhimurium were reduced significantly after 30 min ($P < 0.05$). In particular, *S.* Typhimurium was the most affected by treatment with aerosolized 2% malic acid, which reduced levels of *S.* Typhimurium by 1.54, 2.81, 3.05, and 4.10 log_{10} CFU/g after 10, 30, 50 and 100 min, respectively.

Spinach leaves inoculated with *E. coli* O157:H7 (7.77 log_{10} CFU/g) was significantly reduced by the control treatment (distilled water) after 100 min, but reduction levels were less than 1 log_{10} CFU/g (Table 3). When spinach leaves were treated with aerosolized malic acid, the inhibitory effect increased with increasing concentration of malic acid. Aerosolized 2% malic acid was the most effective concentration for reducing *E. coli* O157:H7 on spinach, resulting in reductions of 1.60, 2.38, 3.18, and 3.67 log_{10} CFU/g after 10, 30, 50 and 100 min, respectively.

Tables 4–6 show levels of surviving cells of *L. monocytogenes*, *S.* Typhimurium and *E. coli* O157:H7 on lettuce before and after treatment with aerosolized distilled water and malic acid. The initial level of *L. monocytogenes* on lettuce leaves was 7.35 log_{10} CFU/g, and was not significantlyreduced by treatment with aerosolized distilled water for 100 min as a control ($P \geq 0.05$) (Table 4). However, when lettuce leaves were treated with aerosolized 0.25 and 0.5% malic acid for 100 min, *L. monocytogenes* was reduced significantly ($P < 0.05$) (0.64 and 1.01 log_{10} CFU/g reduction, respectively). Following treatment with aerosolized 1% and 2% malic acid, *L. monocytogenes* was decreased significantly after 30 min ($P < 0.05$) and reduction levels were 1.21 and 2.17 log_{10} CFU/g, respectively. Also, treatment with aerosolized 2% malic acid for 100 min was the most effective treatment for reducing levels of *L. monocytogenes* (3.85 log_{10} CFU/g reduction).

The initial level of *S.* Typhimurium on lettuce was 8.39 log_{10} CFU/g (Table 5). After treatment with aerosolized distilled water and 0.25% malic acid for 100 min, *S.* Typhimurium was reduced significantly ($P < 0.05$). When lettuce leaves were treated with aerosolized 0.5% malic acid for 50 min, or aerosolized 1 and 2% malic acid for 30 min, levels of *S.* Typhimurium declined significantly ($P < 0.05$). These results show that increasing the concentration of malic acid can decrease the treatment time needed. Among the tested concentrations, treatment with aerosolized 2% malic acid for 100 min reduced *S.* Typhimurium cell numbers the most (5.02 log_{10} CFU/g reduction).

For *E. coli* O157:H7, lettuce leaves were inoculated with 8.23 log_{10} CFU/g (Table 6). We observed less than 1 log_{10} CFU/g reduction in *E. coli* O157:H7 after treatment with aerosolized distilled water for 100 min (control). Unlike *L. monocytogenes* and *S.* Typhimurium, when lettuce leaves were treated with aerosolized 1% and 2% malic acid, *E. coli* O157:H7 was reduced significantly after 10 min ($P < 0.05$). When aerosolized 2% malic acid was applied for 10, 30, 50, and 100 min, the populations of *E. coli* O157:H7 were reduced by 1.38, 2.47, 3.06, and 3.35 log_{10} CFU/g, respectively.

We evaluated the color values (L^*, a^*, and b^*) of the spinach and lettuce before and after treatment with aerosolized water (control),

Table 5
Populations (Log_{10} CFU/g) of *Salmonella* Typhimurium inoculated on lettuce exposed to aerosolized distilled water (control) and malic acid [0.25, 0.5, 1, 2% (w/v)] for 10, 30, 50, and 100 min at room temperature (22 ± 2 °C).

Time (min)	Treatments				
	Water (control)	0.25% Malic acid	0.5% Malic acid	1% Malic acid	2% Malic acid
0	8.39 ± 0.21^{a}	8.39 ± 0.21^{a}	8.39 ± 0.21^{a}	8.39 ± 0.21^{a}	8.39 ± 0.21^{a}
10	8.25 ± 0.31^{ax}	8.03 ± 0.12^{axy}	7.99 ± 0.25^{abxy}	7.74 ± 0.62^{abxy}	7.37 ± 0.48^{ay}
30	7.88 ± 0.59^{abx}	7.78 ± 0.49^{abx}	7.24 ± 0.64^{abcx}	6.79 ± 0.79^{bcxy}	5.84 ± 0.57^{by}
50	7.60 ± 0.40^{abx}	7.61 ± 0.36^{abx}	6.77 ± 1.05^{bcxy}	5.59 ± 1.03^{cdyz}	4.61 ± 0.98^{cz}
100	7.20 ± 0.64^{bx}	7.16 ± 0.65^{bx}	5.91 ± 1.14^{cxy}	4.89 ± 0.85^{dy}	3.37 ± 0.58^{dz}

[a–d]Means with the same letter within a column are not significantly different ($P < 0.05$).
[x–z]Means with the same letter within a row are not significantly different ($P < 0.05$).

Table 6
Populations (Log_{10} CFU/g) of *Escherichia coli* O157:H7 inoculated on lettuce exposed to aerosolized distilled water (control) and malic acid [0.25, 0.5, 1, 2% (w/v)] for 10, 30, 50, and 100 min at room temperature (22 ± 2 °C).

Time (min)	Treatments				
	Water (control)	0.25% Malic acid	0.5% Malic acid	1% Malic acid	2% Malic acid
0	8.23 ± 0.21^{a}	8.23 ± 0.21^{a}	8.23 ± 0.21^{a}	8.23 ± 0.21^{a}	8.23 ± 0.21^{a}
10	8.17 ± 0.19^{axy}	8.12 ± 0.28^{ax}	7.95 ± 0.36^{bxy}	7.22 ± 0.36^{byz}	6.85 ± 0.17^{bz}
30	8.05 ± 0.17^{ax}	7.78 ± 0.33^{ax}	7.41 ± 0.72^{abx}	6.54 ± 0.26^{cy}	5.76 ± 0.39^{cz}
50	7.72 ± 0.50^{abx}	7.12 ± 0.40^{bxy}	6.69 ± 0.59^{bcy}	5.57 ± 0.23^{dz}	5.17 ± 0.23^{cdz}
100	7.27 ± 0.40^{bx}	6.99 ± 0.46^{bx}	6.20 ± 0.50^{cy}	5.38 ± 0.11^{dz}	4.88 ± 0.16^{dz}

$^{a-d}$Means with the same letter within a column are not significantly different ($P < 0.05$).
$^{x-z}$Means with the same letter within a row are not significantly different ($P < 0.05$).

malic acid (0.25. 0.5, 1, 2%) for 100 min during storage at 4 °C. After 7 days, there was no difference on three color values (L^*, a^*, and b^*) between the control (untreated spinach and lettuce) and aerosolized malic acid treated samples indicating treatment with aerosolized malic acid for 100 min did not significantly affect visual color quality of spinach and lettuce.

To date, in the food industry, treatment with aqueous chlorinated water is widely used to reduce foodborne pathogens on fruits and vegetables. However, chlorinated water is of limited efficacy, resulting in microbial reductions of less than 2 log_{10} CFU/g on fresh produce, and exposure of produce to chlorine may produce trihalomethanes (Lee et al., 2004), toxic waste which must be disposed of appropriately. Organic acid could be used as an alternative sanitizer to increase the microbial safety of fresh produce. Previous researchers have studied the lethal activities of aqueous organic acid treatments such as malic acid, acetic acid, lactic acid, and citric acid. Akbas and Ölmez (2007) found that treatment of inoculated iceberg lettuce with 1% lactic acid, citric acid, acetic acid, and ascorbic acid for 5 min reduced *E. coli* O157:H7 by 3.00, 3.10, 2.40, and 2.10 log_{10} CFU/g, and *L. monocytogenes* by 2.20, 1.80, 1.40, and 1.30 log_{10} CFU/g, respectively. Francis and O'Beirne (2002) found that treatment with 1.0% citric acid solution for 5 min reduced mesophilic population densities on lettuce by about 1.50 log_{10} CFU/g Massilia et al. (2009) reported that levels of *L. monocytogenes* and *E. coli* O157:H7 in apple juice was reduced by 1.61 and 1.21 log_{10} CFU/g, respectively, when treated with 1% malic acid. Although treatment with aqueous organic acid was effective at reducing levels of pathogens on fresh produce or juice, they cannot be used during transportation and storage.

Aerosolized sanitizer might be used as an alternative intervention during transportation and storage, since aerosolized dispersal of liquid as a fine mist in air can penetrate remote surfaces of produce enclosed in a confined area. In this study, we investigated effects of aerosolized malic acid in eliminating *L. monocytogenes*, *S.* Typhimurium, and *E. coli* O157:H7 on spinach and lettuce. Treatment with aerosolized 2% malic acid for 100 min proved most effective in the reduction of three pathogens on spinach and lettuce. These results show similar tendency when peroxyacetic acid was applied to aerosolization system. Some researchers have studied the lethal effect of dipping with aqueous peroxyacetic acid to decrease *E. coli* O157:H7 or coliforms on foods. Wang, Feng, and Luo (2006) reported that treatment with 80 ppm peroxyacetic acid for 30 s, 1, 3, and 5 min produced 0.76, 1.21, 1.42, and 1.51 log_{10} CFU/g reductions of *E. coli* O157:H7 on fresh-cut apples. Also, Hilgren and Salverda (2000) noted that levels of coliform bacteria on cabbage were reduced by 0.78 log_{10} CFU/g when treated with 40 ppm peroxyacetic acid for 30 s. Oh et al. (2005) observed that inoculated lettuce leaves treated with aerosolized 40 ppm peroxyacetic acid reduced populations of *E. coli* O157:H7 by 0.80, 2.20, and 3.40 log_{10} CFU/g after 10, 30, and 60 min, respectively. Populations of *S.* Typhimurium were reduced by 0.30, 3.30 and 4.50 log_{10} CFU/g, and *L. monocytogenes* was reduced 2.50, 2.70 and 3.80 log_{10} CFU/g, respectively. Also, Lee, Jung, Jin, Kim, and Oh (2007) studied the effect of aerosolized hydrogen peroxide–based sanitizer against *E. coli* O157:H7 and *L. monocytogenes* on the surface of stainless steel. In their study, when treated with aerosolized 4.0% hydrogen peroxide–based sanitizer for 60 min, *E. coli* O157:H7 was reduced from 9.04 to 0.69 log_{10} CFU/g (8.35 log_{10} CFU/g reduction), and *L. monocytogenes* on the surface of stainless steel was reduced from 8.53 to 0.43 log_{10} CFU/g (8.10 log_{10} CFU/g reduction).

In our study malic acid solution was applied using aerosolization generator to reduce populations of foodborne pathogens on spinach and lettuce. Also, this study was performed to compare the effectiveness of aerosolized malic acid treatments at different treatment concentrations and times for reducing numbers of *L. monocytogenes*, *S.* Typhimurium and *E. coli* O157:H7 on spinach and lettuce. Generally, the degree of reduction increased significantly ($P < 0.05$) with increasing treatment time (from 10 to 100 min) and concentration (from 0.25 to 2%). In particular, aerosolized 2% malic acid was the most effective concentration that reduced *L. monocytogenes*, *S.* Typhimurium, and *E. coli* O157:H7 on spinach and lettuce. These results show that aerosolized sanitizers might be used as an effective way to reduce levels pathogens on fresh produce during transportation and storage. However, according to the study of Lee et al. (2007), the inhibiting effect of aerosolization against foodborne pathogens depends on the sanitizer employed. Aerosolized hydrogen peroxide-based and ammonium-based sanitizers were effective at inhibiting levels of *E. coli* O157:H7 whereas other aerosolized sanitizers such as chlorine-based, iodophor-based, and alcohol-based sanitizers did not significantly reduce levels of *E. coli* O157:H7. Thus, further studies should be performed to improve the effectiveness of various aerosolized sanitizers against pathogenic bacteria on foods. Also, we studied with individual leaves spread in one layer inside the model cabinet. Therefore, further studies should be researched if treating bigger masses of leaves would result in the same level of efficacy.

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References

Akbas, M. Y., & Ölmez, H. (2007). Inactivation of *Escherichia coli* and *Listeria monocytogenes* on iceberg lettuce by dip wash treatments with organic acids. *Journal of Applied Microbiology, 44*, 619–624.

Centers for Disease Control and Prevention. (2006). *E. coli* O157:H7 outbreak from fresh spinach. October 6, 2006 (Accessed October 10, 2007. Available at: http://www.cdc.gov/foodborne.ecolispinach/).

FDA. (2006).Update: *E. coli* O157:H7 outbreak at Taco Bell restaurants likely over FDA traceback investigation continues. http://www.fda.gov.topics/NEWS/2006/NEW01527.html (last accessed 10.08.07).

Francis, G. A., & O'Beirne, D. (2002). Effects of vegetable type and antimicrobial dipping on survival and growth of *Listeria innocua*and *E. coli*. *International Journal of Food Science and Technology, 37*, 711–718.

Hilgren, J. D., & Salverda, J. A. (2000). Antimicrobial efficacy of a peroxyacetic/octanoicacid mixture in fresh-cut-vegetable process waters. *Journal of Food Science, 65*, 1376–1379.

Izat, A. L., Colberg, M., Adams, M. H., Reiber, M. A., & Waldroup, P. W. (1989). Production and processing studies to reduce the incidence of salmonellae on commercial broilers. *Journal of Food Protection, 52*, 670–673.

Lee, S. Y., Yun, K., Fellman, J., & Kang, D. H. (2002). Inhibition of *Salmonell*a Typhimurium and *Listeria monocytogenes* in mung bean sprouts by chemical treatment. *Journal of Food Protection, 65*, 1088–1092.

Lee, S. Y., Costello, M., & Kang, D. H. (2004). Efficacy of chlorine dioxide gas as a sanitizer of lettuce leaves. *Journal of Food Protection, 67*, 1371–1376.

Lee, S. Y., Jung, J. H., Jin, J. H., Kim, Y. H., & Oh, S. W. (2007). Inhibitory effect of aerosolized commercial sanitizers against foodborne pathogens. *Journal of Food Hygiene and Safety, 22*, 235–242.

Massilia, R. M., Melgar, M. J., & Belloso, M. O. (2009). Antimicrobial activity of malic acid against *Listeria monocytogenes, Salmonella Enteritidis* and *Escherichia coli* O157:H7 in apple, pear and melon juices. *Food Control, 20*, 105–112.

Oh, S. W., Dancer, G. I., & Kang, D. H. (2005). Efficacy of aerosolized peroxyaceticacid as a sanitizer of lettuce leaves. *Journal of Food Protection, 68*, 1743–1747.

Park, E. J., Alexander, E., Tarlor, G. A., Costa, R., & Kang, D. H. (2008). .Effect of electrolyzed water for reduction of foodborne pathogens on lettuce and spinach. *Journal of Food Microbiology, 73*, 268–272.

Ricke, S. C. (2003). Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. *Poultry Science, 82*, 632–639.

Venkitanarayanan, K. S., Lin, C. M., Bailey, H., & Doyle, M. P. (2002). Inactivation of *Escherichia coli* O157:H7, *Salmonella enteritidis*, and *Listeria monocytogenes* on apples, oranges, and tomatoes by lactic acid with hydrogen peroxide. *Journal of Food Protection, 65*, 100–105.

Wang, H., Feng, H., & Luo, Y. (2006). Dual-phasic inactivation of *Escherichia coli* O157:H7 with peroxyacetic acid, acidic electrolyzed water and chlorine on cantaloupes and fresh-cut apples. *Journal of Food Safety, 26*, 335–347.