Survival of *Escherichia coli* O157:H7 and *Salmonella enterica* serovars Typhimurium in iceberg lettuce and the antimicrobial effect of rice vinegar against *E. coli* O157:H7

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**Abstract**

The microbiological safety of fresh produce is a significant concern of consumers and industry. After applying at an inoculated level (about 10\(^6\) CFU g\(^{-1}\)) of *E. coli* O157:H7 and *Salmonella enterica* serovars Typhimurium on shredded iceberg lettuce and water samples individually, they were stored at 4\(\, ^\circ\)C for 14 days and 22\(\, ^\circ\)C for 7 days to monitor the growth and survival of pathogens. The results showed that at the end of 4\(\, ^\circ\)C storage, populations of two pathogens in lettuce and water decreased approximately 1 log CFU g\(^{-1}\). However, microbial levels on shredded lettuce increased 3 logs within 3 days at 22\(\, ^\circ\)C. Vinegar (acetic acid) has been used to reduce populations of foodborne pathogens in foods; hence, the antimicrobial effect of rice vinegar on the survival of *E. coli* O157:H7 in inoculated lettuce (10\(^7\) CFU g\(^{-1}\)) is examined in this study. Results were observed that the treatment of inoculated lettuce (10\(^7\) CFU g\(^{-1}\)) with commercial vinegar containing 5\% acetic acid (pH 3.0) for 5 min would reduce 3 logs population at 25\(\, ^\circ\)C. Less than a 1-log decrease in bacterial numbers was recovered during 5 min exposure to 0.5\% (pH 3.26) acetic acid.

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**Keywords:** *Escherichia coli* O157:H7; *Salmonella*; Antimicrobial effect; Vinegar; Lettuce

1. Introduction

The incidence of foodborne infections caused by bacterial pathogens continues to be a problem in industrialized nations and developing countries (Fang, 2005; Lampel et al., 2000). These infections resulted in health and economic burdens in those countries and were especially severe in the young, older and immuno-compromised people (Bailey, 1998). Human infections traditionally are acquired via the ingestion of foods of animal origin which shifted to a vehicle of produce. The number of foodborne outbreaks due to consumption of contaminated fresh produce has increased for three decades (Sivapalasingam et al., 2004; Tauxe et al., 1997). Most of the reported outbreaks were caused by pathogenic bacteria, especially *Escherichia coli* O157:H7 and *Salmonella* (Beuchat, 1996). *E. coli* O157:H7 and *Salmonella enterica* serovars Typhimurium are commonly found in a wide variety of raw meats, dairy products, vegetables (including lettuce) and water (Lang et al., 2004; Rhee et al., 2003; Strachan et al., 2005; Vernozy-Rozand et al., 2005; Wachtel and Charkowski, 2002). Contamination with *E. coli* O157:H7 and *Salmonella* likely occurred on farms through the use of contaminated irrigation water and manure (Bopp et al., 2003; Franz et al., 2005; Johannessen et al., 2005; Sivapalasingam et al., 2004). Enterohemorrhagic *E. coli* O157:H7 is a causative agent of hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). The infection dosage of *E. coli* O157:H7 is as low as 10–100 CFU g\(^{-1}\) (Chiueh et al., 2001; Mccarthy et al., 1998; Shearer et al., 2001). In Taiwan, one of 116 (0.86\%) specimens of fresh-cut vegetables was contaminated with *E. coli* O157:H7...
(Huang et al., 2005). In the US, eight lettuce-associated outbreaks were reported with foodborne pathogens, including *E. coli* O157:H7 and *Salmonella* from 1973 through 1997 (Sivapalasingam et al., 2004). Salmonellosis caused by many *Salmonella* serovars has been found in humans and animals (Lim et al., 2003). The overall rate of infection ranged from 15 to 20/100,000 population, during each year in the US (Oscar, 2004). The outbreaks caused by *Salmonella* spp. were frequent, both in Korea (20.7%) and in Japan (14.2%) (Lee et al., 2001). *S. enterica* serovars Typhimurium and *S. enterica* serovar Enteritidis are the most frequently isolated serovars from foodborne outbreaks throughout the world (Lim et al., 2003; Tsen, 2002).

Chlorinated water (50–200 ppm) is widely used to sanitize whole fruits and vegetables as well as fresh-cut produce on a commercial scale. However, studies have shown that treatment of produce with chlorinated water has a limited bactericidal effect (Mcwatters et al., 2002). Hence, other sanitizers such as organic acids have been studied for their effectiveness to increase food microbiology safety (Sengun and Karapinar, 2004). Organic acids are weak acids that are generally considered more effective against foodborne pathogens than inorganic acids such as hydrochloric acid (Buchanan et al., 2004). Generally speaking, the antimicrobial species of organic acids are fully protonated species which can diffuse into the bacterial cell and cause cell death (Bjornsdottir et al., 2006; Brul and McClure, 2002). These organic acids, including: acetic acid, propionic acid, lactic acid, and citric acid, which are naturally found in a variety of fruits and fermented foods, all belong to the category of GRAS for additives (Fang and Hsueh, 2000). Vinegar (acetic acid) has been studied for its effectiveness in removing pathogens from fresh fruits and vegetables (Rhee et al., 2003; Wu et al., 2000). One of the characteristics common among enterohemorrhagic *E. coli* (EHEC) is their relative tolerance to acidic environments (Buchanan et al., 2004; Fang and Hsueh, 2000; Mccarthy et al., 1998). Acidic or fermented foods, including: apple cider, salami and apple juice have been associated with outbreaks of disease caused by *E. coli* O157:H7 (Bjornsdottir et al., 2006; Han and Linton, 2004; Rhee et al., 2003).

The bacterial foodborne outbreaks associated with fresh produce have compelled the government and industry to increase their efforts for ensuring the highest microbiological quality of domestic and imported fresh produce. Nevertheless, the individual survival and growth behavior of *E. coli* O157:H7 and *S. enterica* serovars Typhimurium on iceberg lettuce and water have not been frequently reported. Many factors affect the antimicrobial activity of organic acids, including: pH, acid concentration, bacterial strains and environment (Bjornsdottir et al., 2006). In this study, the effects of rice vinegar (fermented from rice) on inoculated lettuce for decreasing the *E. coli* O157:H7, were examined first.

The present study was designed to (1) investigate the survival and growth behavior of *E. coli* O157:H7 and *S. enterica* serovars Typhimurium inoculated on lettuce and water at 4 and 22 °C, and between 7 and 28 days; (2) examine the antimicrobial effect of rice vinegar on chopped lettuce with the inoculum (10⁴ and 10⁵ CFU g⁻¹) of *E. coli* O157:H7 at 25 °C.

2. Materials and methods

2.1. Lettuce investigated

Fresh iceberg lettuce (*Lactuca sativa*) samples were purchased from a local supermarket in Taichung City, Taiwan, ROC. The outer leaves and core were removed aseptically and discarded. The specimens of lettuce exhibited the absence of *E. coli* O157:H7, *Salmonella*, and low aerobic plate count of lettuce, i.e. about 5 log CFU g⁻¹. All lettuces were kept at 2–5 °C between the time of purchase and initiation of experiments and were then used immediately.

2.2. Bacterial strains

*E. coli* O157:H7 BCRC 13086 and *S. enterica* serovars Typhimurium ATCC 14028 strains were obtained from the Biosources Collection and Research Center (BCRC) of Food Industry Research and Development Institute, Hsinchu, Taiwan. Each isolate was grown in tryptic soy broth was streak-cultured on Sorbitol MacConkey agar (SMAC) (Merck) surfaces containing cefixime (0.05 mg l⁻¹), potassium tellurite (2.5 mg l⁻¹) and Levine’s Eosin-Methylene blue agar (LEMB), and then incubated overnight at 37 °C (Shearer et al., 2001). Non-sorbitol-fermenting colonies on CT-SMAC (Merck) surfaces containing cefixime (0.05 mg l⁻¹), potassium tellurite (2.5 mg l⁻¹) and Levine’s Eosin-Methylene blue agar (LEMB), and then incubated overnight at 37 °C (Shearer et al., 2001). Non-sorbitol-fermenting colonies on CT-SMAC and black–violet colonies on L-EMB were inoculated to triple sugar iron (TSI) (Merck, Darmstadt, Germany) and TSA, for cultivation at 37 °C for 18 h. The isolates were confirmed...
by using Vitek 32 system with a GNI card (Biomerieux, Inc., USA) for biochemical tests and agglutination with O157 and H7 antisera (Denka Seiken Co., LTD. Tokyo, Japan).

The detection methods of *Salmonella* spp. were based on a previous report (Chang and Chen, 2003). For detection of *Salmonella* spp., 25 g of shredded lettuce were mixed with 225 ml of lactose broth (LB) (Merck, Darmstadt, Germany) to make a 10-fold enrichment broth. The broth was inoculated at 37 °C for 18 h. One ml LB was added to 10 ml tetrathionate broth (TT) (Merck) for secondary enrichment at 37 °C for 18 h. The above broths were streak-cultured on duplicate Hektoen enteric agar (HE) (Difco, Becton Dickinson and Company, USA) and Xylose-lysine-deoxycholate agar (XLD) (Difco, Becton Dickinson and Company, USA) and inoculated at 37 °C for 18 h. The suspected colony was inoculated to TSI and TSA for cultivation at 37 °C for 18 h. The above broths were streak-cultured on duplicate Hektoen enteric agar (HE) (Difco, Becton Dickinson and Company, USA) and Xylose-lysine-deoxycholate agar (XLD) (Difco, Becton Dickinson and Company, USA) and inoculated at 37 °C for 18 h. The suspected colony was inoculated to TSI and TSA for cultivation at 37 °C for 18 h.

Vitek 32 system with a GNI card (Biomerieux, Inc., USA) was used for biochemical tests, and serological typing was confirmed with *Salmonella* O-antisera from Denka Seiken (Tokyo, Japan).

### 2.4. Preparation of inoculum and inoculation of lettuce and water

Two reference strains were cultured on a TSA plate overnight at 37 °C preceding the experiments. Several colonies were suspended in sterile water to achieve a McFarland 1.0 (about 10⁶ CFU ml⁻¹) by a Vitek colorimeter (Biomerieux Vitek, Inc., USA). The lettuce samples were shredded into 10–15 mm pieces with a sterile knife, and placed into a 2000 ml sterile beaker. One sterile beaker of lettuce (100 g) was inoculated with 4 ml of 10⁶ CFU ml⁻¹ of *E. coli* O157:H7 and the other beaker of lettuce (100 g) was inoculated with 4 ml of 10⁶ CFU ml⁻¹ of *S. enterica* serovars Typhimurium for 10 min to achieve a final level (approximately 10⁶ CFU g⁻¹) of each pathogen. Ten g of inoculated lettuce were separately placed in a 50 ml sterile centrifuge tube and stored at 4 and 22 °C for 0–14 days. 100 ml of sterilized water was inoculated with 1 ml of 10⁶ CFU ml⁻¹ of *E. coli* O157:H7 and *S. enterica* serovars Typhimurium for 10 min to achieve a final level 10⁶ CFU g⁻¹ of each pathogen. Ten ml of inoculated water was separately placed into a 50 ml sterile centrifuge tube and stored at 4 and 22 °C for 0–28 days. Un-inoculated lettuce and sterile water were used as controls.

### 2.5. Procedures for enumeration of microorganisms

Five selective medium were used for the identification and enumeration of *E. coli* O157:H7 and *S. enterica* serovars Typhimurium in lettuce. The color of suspected colonies for isolation of *Salmonella* in XLD medium was red. On HE medium, the suspected colonies of *Salmonella* were blue–green in color. The black–violet and gray–white colors of colonies were suspected for isolation of *E. coli* O157:H7 in L-EMB and CT-SMAC, respectively. However, the Luria-Bertani agar (LB) (Difco, Becton Dickinson and Company, USA) was used for enumeration of total aerobic counts (all colonies in yellow to white color).

Ten g of inoculated lettuce were removed from the storage container and added to 90 ml of 0.85% saline to make a 10-fold dilution. Then, 1 ml of 10-fold dilution was mixed with 9 ml of 0.85% saline and prepared in a complete dilution series from 10⁻² to 10⁻⁴. We then spread 0.1 ml of serial dilution (10⁻²–10⁻⁴) on duplicate CT-SMAC, L-EMB and LB plates for detection of *E. coli* O157:H7 and on duplicate XLD, HE as well as LB plates for detection of *S. enterica* serovars Typhimurium. Those plates were inoculated at 37 °C for 18 h, and the suspected colonies of the pathogens were counted. Three presumptive colonies were cultivated to TSA and TSI (Merck, Darmstadt, Germany) at 37 °C for 18 h and confirmed by a Vitek 32 system for biochemical test; serological typing was confirmed with *Salmonella* O-antisera and *E. coli* O157 antigen from Denka Seiken (Tokyo, Japan).

Inoculated water was sampled at 0, 3, 7, 14, 20, and 28 days for observation of the presence of each pathogen at 4 and 22 °C. Ten ml of inoculated water was removed from the storage container and added to 90 ml of 0.85% saline to make a 10-fold dilution. One ml of 10-fold dilution was mixed with 9 ml of 0.85% saline, and we prepared a complete dilution series from 10⁻² to 10⁻⁴. We then spread 0.1 ml of serial dilution onto surfaces of LB plates in duplicate for enumeration of *Salmonella* and *E. coli* O157:H7. Those LB plates were inoculated at 37 °C for 24 h, and enumerated.

### 2.6. Treatment of rice vinegar

The rice vinegar (Tan An Kong Yen Food Co., Ltd., Taiwan) containing 5% (v/v) acetic acid was purchased at a local supermarket in Taichung City. The commercial vinegar containing 5% acetic acid (pH 3.0) was diluted to vinegar solutions 0.05% acetic acid (pH 4.09) and 0.5% acetic acid (pH 3.26) with sterile water. The sterilized distilled water was used as control. Various concentrations of vinegar (0%, 0.05%, 0.5% and 5% acetic acid) were used to treat inoculated chopped lettuce.

The treatment procedure was modified from the previous study for investigating the survival of *E. coli* O157:H7 in shredded lettuce (Wu et al., 2000). First, 60 g of lettuce were individually inoculated with approximately 3 ml of *E. coli* O157:H7 (10⁸ and 10⁶ CFU ml⁻¹) to attain an inoculated level of *E. coli* O157:H7 (10⁸ and 10⁶ CFU g⁻¹), and then was homogenized for 10 min. Then, 10 g of inoculated lettuce (10⁸ and 10⁶ CFU g⁻¹) were mixed with 40 ml of four vinegar treatment solutions (0%, 0.05%, 0.5% and 5% acetic acid) in a sterile plastic bag for 5 min at 25 °C. Finally, 1 ml of the treated specimen was added to 9 ml BHI to make 10-fold dilution in series (10⁻¹–10⁻⁵). 100 μl of serial diluted specimen was spread on duplicate CT-SMAC, L-EMB and LB plates. Those plates were...
inoculated at 37 °C for 24 h. The suspected colonies were enumerated and cultivated to TSA and TSI at 37 °C for 24 h and confirmed by biochemical and serological test, as described above.

2.7. Statistical analysis of data

The data from the independent replication trials were analyzed statistically by using the General Linear Models procedures of SAS (SAS Institute, 1989, Statistical Analysis System, Cary, NC, USA). Significant differences between mean populations of pathogen were determined using Duncan’s Multiple Range Test. Repeated measurement tests were used to determine the significant difference among treatments. Significance was expressed at 5% or 1% level.

3. Results

3.1. Survival and growth of E. coli O157:H7 and S. Typhimurium in shredded lettuce and water

Shredded lettuce inoculated with 10^6 CFU g^-1 of E. coli O157:H7 and S. Typhimurium were monitored for changes in pathogenic populations during storage at 4 and 22 °C for up to 14 days. Investigation of inoculated lettuce was terminated when visual examination revealed that lettuce was inedible, i.e. the tissue began to liquefy and develop an off odor. At 4 °C, populations of the pathogens on chopped lettuce declined approximately 1 log throughout the 14-day storage (Table 1). For E. coli O157:H7, the populations declined from 6.65 to 5.26 log CFU g^-1. For S. Typhimurium, populations declined from 6.40 to 5.30 log CFU g^-1. Unlike the decrease in populations of two pathogens on shredded lettuce at 4 °C, populations of the E. coli O157:H7 on shredded lettuce increased from 6.65 to 9.36 log CFU g^-1, and S. Typhimurium from 6.40 to 9.26 log CFU g^-1, respectively, within 3 days storage at 22 °C.

The survival and growth rates of the two pathogens, in water at 4 and 22 °C for 28 days, are shown in Table 2. At the end of 4 °C storage, the presence of E. coli O157:H7 declined from 5.97 to 4.93, and S. Typhimurium declined from 5.75 to 5.11 log CFU g^-1, respectively. However, the survival levels of E. coli O157:H7 decreased from 6.48 to 5.18 log CFU g^-1 and S. Typhimurium from 6.23 to 5.20 log CFU g^-1 at the end of 22 °C storage. The results revealed that the population of two pathogens decreased 1 log in water after 28 days at 4 and 22 °C.

3.2. Survival of E. coli O157:H7 on lettuce treated with rice vinegar for 5 min at 25 °C

The population of E. coli O157:H7 was counted in inoculated lettuce samples without rice vinegar treatment as control in this study. The effectiveness of rice vinegar, containing 0.05% acetic acid (pH 4.09) and 0.5% acetic acid (pH 3.26) (v/v), on E. coli O157:H7 inoculated lettuce samples at high (7 log CFU g^-1) and low (4 log CFU g^-1) inoculum levels is presented in Fig. 1. The result showed

**Table 1** Survival and growth of E. coli O157:H7 and S. Typhimurium in lettuce

<table>
<thead>
<tr>
<th>Species</th>
<th>log CFU g^-1</th>
<th>4 °C (days)</th>
<th>22 °C (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0  3  7  14</td>
<td>0  2  3  7</td>
</tr>
<tr>
<td>E. coli O157:H7a</td>
<td>6.65 ± 0.36</td>
<td>6.48 ± 0.32</td>
<td>6.20 ± 0.28</td>
</tr>
<tr>
<td>S. Typhimuriumd</td>
<td>6.40 ± 0.28</td>
<td>5.72 ± 0.37</td>
<td>5.65 ± 0.26</td>
</tr>
</tbody>
</table>

^a Mean values from independent experiments (n = 3). ^b Population was counted on CT-SMAC plates. ^c Not performed because of lettuce deterioration. ^d Population was counted on XLD plates.

**Table 2** Survival and growth of E. coli O157:H7 and S. Typhimurium in water

<table>
<thead>
<tr>
<th>Species</th>
<th>log CFU g^-1</th>
<th>4 °C (days)</th>
<th>22 °C (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0  3  7  14</td>
<td>0  3  7  14 28</td>
</tr>
<tr>
<td>E. coli O157:H7</td>
<td>5.97 ± 0.37</td>
<td>5.40 ± 0.27</td>
<td>5.38 ± 0.33</td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>5.75 ± 0.33</td>
<td>5.62 ± 0.27</td>
<td>5.53 ± 0.34</td>
</tr>
</tbody>
</table>

^a Mean values from independent experiments (n = 3). ^b Population was counted on LB plates.
that commercial rice vinegar dilutions containing 0.05% acetic acid (pH 4.09) and 0.5% acetic acid (pH 3.26), reduced less than 1 log CFU g\(^{-1}\) on chopped lettuce after 5 min exposure. Results of using rice vinegar containing 5% (v/v) acetic acid on \textit{E. coli} O157:H7 inoculated lettuce samples at high (7 log CFU g\(^{-1}\)) and low (4 log CFU g\(^{-1}\)) inoculum levels are presented in Fig. 1. Populations of \textit{E. coli} O157:H7 in high inoculum lettuce were reduced by 3 logs CFU g\(^{-1}\) after 5 min treatments, and in low inoculum were reduced to an undetectable level (<1 log CFU g\(^{-1}\)).

4. Discussion

4.1. Survival of \textit{E. coli} O157:H7 and \textit{S. Typhimurium} on shredded lettuce at 4 and 22 \textdegree{}C

Growth and survival of \textit{E. coli} O157:H7 and \textit{S. Typhimurium} on shredded lettuce within shelf life (10–12 days) revealed that survivability of these pathogens imposed a potential risk to consumers. Our findings indicate that survival of \textit{E. coli} O157:H7 and \textit{S. Typhimurium} decreased approximately 1 log CFU g\(^{-1}\) at 4 \textdegree{}C during 14 days of storage and that the lettuce samples increased 3 logs within 3 days at 22 \textdegree{}C, suggesting that the pathogens were able to survival in lettuce at 4 and 22 \textdegree{}C. The previous report (Huang et al., 1998) had studied the presence of \textit{E. coli} O157:H7 on salad vegetables. Populations of viable \textit{E. coli} O157:H7 declined on vegetables stored at 5 \textdegree{}C, and increased on vegetables stored at 21 \textdegree{}C for up to 14 days. The populations of \textit{E. coli} O157:H7 increased most rapidly on lettuce and cucumbers during storage at 21 \textdegree{}C (Abdul-Raouf et al., 1993). Survival and growth of \textit{Shigella flexneri} in cooked rice, lentil soup, cooked fish, mashed potato and raw cucumber was studied in an early report. The pathogen grew well in all tested foods, and growth increased from 10\(^5\) to 10\(^8\) CFU ml\(^{-1}\) or CFU\(^{-1}\) within 18 h after inoculation at 25 \textdegree{}C (Islam et al., 1993).

4.2. Survival of \textit{E. coli} O157:H7 and \textit{S. Typhimurium} in water at 4 and 22 \textdegree{}C

The previous studies (Johannessen et al., 2005; Kudva et al., 1998; Wang et al., 1996) indicated that \textit{E. coli} could survive for extended periods in manure and water. A number of \textit{E. coli} O157:H7 outbreaks have been linked to contaminated water; moreover, studies (Solomon et al., 2002) have demonstrated the ability of the pathogen to survive for extended periods in water. In this study, results indicate that the survival and growth of \textit{E. coli} O157:H7 and \textit{S. Typhimurium} in water declined 1 log at 4 and 22 \textdegree{}C after 28 days storage. It was reported that the presence of \textit{Shigella flexneri} in distilled water decreased gradually until the final count of 26 days (Rafii and Lunsford, 1997). After 26 days, the bacterial count was 9.20 \times 10\(^7\) CFU ml\(^{-1}\) (32.8% survival). In addition, by chilling and washing with contaminated water, produce contamination can occur during post-harvest processing. Therefore, the long survivability of \textit{E. coli} O157:H7 and \textit{S. Typhimurium} in water at 4 and 22 \textdegree{}C could constitute a threat to public health.

4.3. The antimicrobial effect of rice vinegar on chopped lettuce with the inoculum (10\(^4\) and 10\(^7\) CFU g\(^{-1}\)) of \textit{E. coli} O157:H7 at 25 \textdegree{}C

Acidified products may limit microbial growth or survival, depending on the types of microorganisms harbored in the food and the type and amount of acid, especially its buffering capacity (Bjornsdottir et al., 2006). The results in our study indicate that commercial rice vinegar (5.0%) could reduce 3 log population of \textit{E. coli} O157:H7 in iceberg lettuce at 25 \textdegree{}C while low concentration of vinegar (0.5% and 0.05%) was not found to exhibit any antimicrobial effect. In the previous study (Fang and Tsai, 2003), acetic acid was found to be the most effective agent in the inhibition of the growth of \textit{E. coli} in ground beef stored at 10 and 30 \textdegree{}C. It was reported that acetic acid was the most lethal acid to \textit{E. coli} O157:H7, followed by lactic, citric, and malic acids, in testing over a range of pH values (Ryu et al., 1999). The early study suggested that using 5.0% (v/v) acetic acid for 5 min at 21 \textdegree{}C can reduce the population of \textit{Shigella sonnei} on parsley more than 6 log CFU g\(^{-1}\) (Wu et al., 2000). As for the effects of lemon juice and vinegar (1:1) mixture at high (5.64 log CFU ml\(^{-1}\)) and low (2.58 log CFU ml\(^{-1}\)) inoculum levels of \textit{S. Typhimurium}, the initial populations were reduced to an undetectable level after 30 min treatment (Sengun and Karapinar, 2004).

Vijayakumar and Wolf-Hall (2002) reported that iceberg lettuce treated with the white vinegar for 10 min were noticeably sour and slightly wilted in appearance. Nevertheless, consumer acceptability was maintained with all
sanitization treatments, including those involving 35% white vinegar (1.9% acetic acid). Wu et al. (2000) pointed that parsley treated with vinegar containing ≥2.6% acetic acid noticeably discolored and had a strong vinegar odor. In our study, the addition of 5.0% of rice vinegar may give lettuce an unacceptable sour flavor; however, washing with tap water will improve the unacceptable flavor. The relation between the antimicrobial effect of rice vinegar and consumer acceptance requires further study.

Overall, the results in our study revealed the survival characteristics of E. coli O157:H7 and S. Typhimurium on the surface of lettuce, and water stored at 4 and 22 °C for 7–28 days. At 4 °C, populations of the pathogen organisms on chopped lettuce declined 1 log CFU g⁻¹ throughout the 14-day storage. Nevertheless, two pathogens could survive and decrease less than 1 log for 28 days at 4 and 22 °C in water. Rice vinegar with various diluted solutions was tested for effectiveness in reducing counts of inoculated E. coli O157:H7 on lettuce. Treatment of inoculated lettuce with rice vinegar might offer a useful method to decrease the risk of E. coli O157:H7 infection either in restaurants or at home. The results in this study indicate that treatment with 5% acetic acid (pH 3.0) for 5 min at 25 °C could reduce 3 log CFU g⁻¹ of E. coli O157:H7 on chopped lettuce initially inoculated with 7 log CFU g⁻¹. In addition, treatment of chopped lettuce with 5% acetic acid would decrease the initial inoculum (4 log CFU g⁻¹) to an undetectable level (<1 log CFU g⁻¹). Interestingly, vinegar at a concentration range from 0.5% (pH 3.26) to 0.05% (pH 4.09) revealed no inhibitory effect in eliminating E. coli O157:H7 on lettuce, in this study.

Acknowledgment

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Bailey, J.S., 1998. Detection of Salmonella cells within 24–26 hours in white vinegar (1.9% acetic acid). Wu et al. (2000) pointed that parsley treated with vinegar containing ≥2.6% acetic acid noticeably discolored and had a strong vinegar odor. In our study, the addition of 5.0% of rice vinegar may give lettuce an unacceptable sour flavor; however, washing with tap water will improve the unacceptable flavor. The relation between the antimicrobial effect of rice vinegar and consumer acceptance requires further study.

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