Short communication

Efficacy of chlorine for inactivation of *Escherichia coli* on vegetables

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Received 27 September 1999; accepted 21 February 2000

Abstract

The effectiveness of calcium hypochlorite on inactivation of *Escherichia coli* inoculated on fresh produce was investigated. Different exposure times and concentrations of chlorine were studied. Dipping was not effective at eliminating *E. coli* populations although it significantly reduced the *E. coli* counts compared with inoculated, undipped lettuce. Dipping inoculated cos lettuce leaves into hypochlorite solutions containing 50 mg/l or greater free chlorine for times of 30 s or greater reduced *E. coli* cells by approximately 1.9–2.8 log_{10} CFU/g from an initial population of approximately 6.8 log_{10} CFU/g. Dipping inoculated broccoli florets into hypochlorite solution reduced *E. coli* cells by approximately 1.7–2.5 log_{10} CFU/g, depending on the time and concentration of the free chlorine. Dipping lettuce or broccoli in water alone reduced cell numbers by 1.5–1.8 log_{10} CFU/g. Dipping broccoli florets for 2 min in a 100 mg/l free chlorine solution at temperatures between 4 and 25°C reduced *E. coli* cells by approximately 2.4 log_{10} CFU/g. No significant effect of temperature on the rate of cell reduction was observed. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Chlorine; *E. coli*; Broccoli; Cos lettuce

1. Introduction

There have been an increasing number of outbreaks of food poisoning linked to the consumption of vegetables and fruit in Westernised countries. The number of reported outbreaks in the USA more than doubled from the period 1973–1987 to the period 1988–1991 (Tauxe et al., 1997). Incidences of disease associated with bacteria have been attributed to *Escherichia coli*, *Salmonella*, *Listeria*, *Shigella*, *Bacillus*, *Aeromonas*, *Clostridium* and *Campylobacter* (Beuchat, 1995; Fain, 1996; Little et al., 1997).

Hypochlorite dips are commonly used postharvest for sanitising fruits and vegetables, particularly in the fresh-cut industry. Washing reduces the total microbial load and in so doing reduces spoilage and maintains quality. The effect of chlo-
rination on reducing postharvest spoilage microorganisms is well known. However, its effect on potential human pathogens is not well understood.

Of the few studies that have been carried out, most have investigated the effect of chlorine on the inactivation of *Listeria monocytogenes*. Chlorine washing was found to reduce *L. monocytogenes* populations on lettuce and Brussels sprouts by only 2 log_{10} CFU/g or less (Brackett, 1987; Beuchat and Brackett, 1990; Zhang and Farber, 1996). Zhuang et al. (1995) looked at the effect of chlorine on *Salmonella montevideo* inoculated on tomatoes. Chlorine was found to reduce populations by around 1 log_{10} CFU/g.

The presence of the coliform *E. coli* is often used as an indicator of faecal contamination. There have been several human disease outbreaks associated with fresh produce caused by enterotoxigenic and enterohaemorrhagic *E. coli* and other faecal organisms such as *Salmonella*, and *Campylobacter* (Beuchat, 1995; Little et al., 1997). Increasingly pressure is being placed on primary producers to ensure that produce is safe for human consumption. The introduction of HACCP-based quality assurance programs and the emphasis on food safety has meant that chlorination is used more and more as a tool to satisfy HACCP requirements. The main aim of this study was to look at the effective of various chlorine concentrations and contact times on the fate of *E. coli* inoculated on fresh produce.

2. Materials and methods

2.1. Preparation of the *E. coli* suspension

*E. coli* (strain TGI) were used for this study. The *E. coli* were cultured each week on luria-bertani (LB) agar (containing 1 µl/ml ampicillin). For each experiment, one loopful of the culture was inoculated into a flask with LB and incubated with shaking for 24 h at 35°C. The concentration of this stock suspension was confirmed by making serial dilutions in peptone buffer containing 0.1% bacto peptone (Difco, Detroit, USA) in deionised water. These dilutions were plated onto Petrifilm E. coli/coliform count plates (3M Australia, NSW) and incubated for 24 h at 35°C.

2.2. Preparation of the hypochlorite solutions

Hypochlorite solutions were prepared using calcium hypochlorite (650 g/kg available chlorine, Premium Quality Pool Products, NSW, Australia) and deionised water. Chlorine solutions (50 and 100 mg/l) were prepared and the pH adjusted to 6.0–6.5 by addition of 1% citric acid solution (Sigma, NSW, Australia). These solutions and a control solution of deionised water were cooled to 4°C. The concentrations of free chlorine were measured using a Hach DR/2000 meter and found to be 45–46 mg/l and 78–83 mg/l for the 50 and 100 mg/l solutions, respectively.

2.3. Effect of chlorine on *E. coli* inoculated on cos lettuce

Fresh cos lettuce (*Lactuca sativa*) was purchased from a local produce market and cooled overnight to 4°C. The older, damaged, outer leaves were discarded and the leaves used were cut in half transversely to enable ease of dipping. An inoculation solution of the *E. coli* was prepared by dilution of the stock suspension in peptone buffer (0.1%) to make 2 l and the concentration determined. Approximately 100 g of the leaves were placed into the inoculation suspension for 1 min. They were removed, the excess solution shaken off and then dipped into 2 l of either the 50, 100 mg/l chlorine solutions or deionised water for 30 s, 2 or 5 min. This inoculation and dipping model represents fresh or recent contamination of the produce. In theory this type of contamination would be easier to remove as there is no time for adherence of the bacteria to the produce surface or to biofilms. Four replicates of each treatment were carried out and fresh solutions were used for each sample. The order of dipping was carried out according to a randomised complete block design to allow for any variations which might occur during the course of the experiment. Following dipping, the samples were weighed into sterile bags and stomached for 2 min in 225 ml of peptone buffer (0.1%). Serial dilutions in peptone
buffer were prepared and plated as described above. This experiment was carried out 3 times, twice using a high inoculum of approximately 7.3 log_{10} CFU/ml and once using a low inoculum of 2.64 log_{10} CFU/ml. For the second high inoculum experiment 6 l of chlorine/water dip were used for each sample to enable agitation via a magnetic stirrer, with three replicates of each treatment. The concentration of _E. coli_ on inoculated but undipped lettuce was determined on two replicates that were included in the block design. The concentration of _E. coli_ on fresh lettuce (not inoculated or dipped) was also determined in duplicate.

2.4. Effect of chlorine on _E. coli_ inoculated on broccoli florets

Fresh broccoli (Brassica oleracea, Botrytis Group) was purchased from a local produce market and cooled overnight to 4°C. It was then processed manually into florets. An inoculation solution was prepared as described for cos lettuce. Approximately 50 g of florets were dipped in 2 l as above according to a randomised complete block design and the concentration of surviving _E. coli_ determined as above. This experiment was carried out twice, once using a high inoculum of 6.68 log_{10} CFU/ml and once using a low inoculum of 2.62 log_{10} CFU/ml. During both experiments the broccoli was agitated during subsequent chlorine/water dipping using a magnetic stirrer. The concentration of _E. coli_ on inoculated but undipped broccoli was determined on three replicates that were included in the block design. The concentration of _E. coli_ on fresh broccoli (not inoculated or dipped) was also determined on three replicates.

2.5. Dip temperature effects

Inactivation of _E. coli_ inoculated onto broccoli florets was determined at 4, 8, 15, 20 and 25°C dip temperatures using 100 mg/l chlorine for 2 min. An inoculation solution was prepared as described and approximately 50 g of florets were dipped. Four replicates of each treatment were carried out. The inoculum contained 7.16 log_{10} CFU/ml. The order of dipping was carried out according to a randomised complete block design and the concentration of surviving _E. coli_ determined as above. The concentration of _E. coli_ on inoculated but undipped broccoli was determined on four replicates that were included in the block design. The concentration on fresh broccoli (not inoculated or dipped) was also determined in duplicate.

2.6. Data analysis

Analysis of variance (GENSTAT 5.4.1) was performed on log_{10} _E.coli_ count (or log_{10} (_E coli_ count + 1)) data to meet the assumption of constant variance. The low inoculum broccoli experiment was not analysed as only seven experimental units had non-zero counts. The treatments were partitioned as a factorial structure of contact time \times chlorine concentration plus an extra treatment (undipped inoculated lettuce or broccoli) where appropriate, in a randomised complete block design. For the high inoculum broccoli experiment, linear effects of chlorine concentration \times contact time were also tested. In the experiments where the undipped inoculated product were missing one or two replicates, the appropriate least significant difference (LSD) for comparing this treatment to the dip treatments was used. It was not presented in the tables for simplicity, as the LSD is only marginally larger than the one presented and the treatment effects are large in comparison. All tests were at the 5% significance level.

3. Results

3.1. Effect of chlorine on inactivation of _E. coli_ inoculated on cos lettuce

The population of _E. coli_ recovered from the cos lettuce following inoculation was 0.5–0.7 log_{10} counts less than that in the suspension (Table 1). Dipping was not effective at eliminating _E. coli_ populations although it significantly reduced the _E. coli_ counts compared with inoculated, undipped lettuce. When lettuce was inoculated
Table 1
Survival of *E. coli* on cos lettuce leaves dipped in chlorine solutions (pH 6.0–6.5)*\(^{a,b}\)

<table>
<thead>
<tr>
<th>Free chlorine (mg/l)</th>
<th>Contact time</th>
<th>Experiment 1</th>
<th></th>
<th>Experiment 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean log(_{10}) CFU/g</td>
<td></td>
<td>Mean log(_{10}) CFU/g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 s</td>
<td>2 min</td>
<td>5 min</td>
<td>Mean</td>
<td>30 s</td>
</tr>
<tr>
<td>0</td>
<td>5.30</td>
<td>5.24</td>
<td>5.22</td>
<td>5.25</td>
<td>5.21</td>
</tr>
<tr>
<td>50</td>
<td>4.60</td>
<td>4.61</td>
<td>4.30</td>
<td>4.50</td>
<td>4.63</td>
</tr>
<tr>
<td>100</td>
<td>4.40</td>
<td>4.19</td>
<td>4.31</td>
<td>4.30</td>
<td>4.65</td>
</tr>
</tbody>
</table>

LSD (\(P = 0.05\))

<table>
<thead>
<tr>
<th></th>
<th>Experiment 1</th>
<th></th>
<th>Experiment 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No dip</td>
<td>6.97</td>
<td></td>
<td>6.55</td>
<td></td>
</tr>
<tr>
<td>LSD ((P = 0.05))</td>
<td>0.38</td>
<td></td>
<td>0.22</td>
<td></td>
</tr>
</tbody>
</table>

* Experiment 1 was without agitation of the lettuce in the dip whereas in experiment 2 the lettuce was agitated during dipping.

* Concentration of inoculating suspension: experiment 1 = 7.32 log\(_{10}\) CFU/ml, experiment 2 = 7.28 log\(_{10}\) CFU/ml. *E. coli* were not detected on uninoculated, undipped lettuce.

with 7.3 log\(_{10}\) CFU *E. coli*/ml, lettuce dipped in solutions with 50 or 100 mg/l chlorine had significantly reduced *E. coli* counts compared with deionised water (Table 1). Dipping cos lettuce leaves into deionised water reduced *E. coli* cells by approximately 1.7 log\(_{10}\) CFU/g compared with inoculated, undipped lettuce. Dipping lettuce leaves into a chlorine solution containing 50 mg/l or greater free chlorine for at least 30 s reduced *E. coli* cells by between 1.9 and 2.8 log\(_{10}\) CFU/g. Agitation of the lettuce during dipping did not appear to affect *E. coli* populations compared with not agitating the lettuce, since reduction of populations was similar in the two experiments. At the lower inoculum concentration (2.7 log\(_{10}\) CFU *E. coli*/ml), lettuce dipped in solution with 100 mg/l chlorine had significantly reduced *E. coli* counts compared with deionised water (data not shown). *E. coli* cells were reduced by between 1.3 and 1.8 log\(_{10}\) CFU/g by dipping in 50–100 mg/l free chlorine for 30 s or longer. *E. coli* were not detected on uninoculated, undipped lettuce.

3.2. Effect of chlorine on inactivation of *E. coli* inoculated on broccoli florets

The population of *E. coli* recovered from the broccoli following inoculation was 1 log\(_{10}\) count lower than that in the suspension (Table 2). Dipping was not effective at eliminating *E. coli* populations although it significantly reduced the *E. coli* counts compared with inoculated, undipped broccoli. Dipping inoculated broccoli florets into deionised water or chlorine reduced *E. coli* cells by between 1.7 and 2.5 log\(_{10}\) CFU/g (Table 2) compared with the undipped broccoli. There was no significant difference between water and chlorine after the 30 s dip. For the 2 and 5 min dip times, a significant linear decrease was observed as the chlorine concentration was increased. There

Table 2
Survival of *E. coli* on broccoli florets dipped in chlorine solutions (pH 6.0–6.3)*\(^{a}\)

<table>
<thead>
<tr>
<th>Free chlorine (mg/l)</th>
<th>Contact time</th>
<th>Mean log(_{10}) CFU/g</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30 s</td>
<td>2 min</td>
</tr>
<tr>
<td>0</td>
<td>3.75</td>
<td>3.96</td>
<td>3.60</td>
</tr>
<tr>
<td>50</td>
<td>3.62</td>
<td>3.44</td>
<td>3.26</td>
</tr>
<tr>
<td>100</td>
<td>3.82</td>
<td>3.17</td>
<td>2.98</td>
</tr>
</tbody>
</table>

LSD (\(P = 0.05\))

|                | 5.52 | 0.35 |

* Concentration of inoculating suspension: 6.68 log\(_{10}\) CFU/ml. *E. coli* were not detected on uninoculated, undipped broccoli.
Table 3
Effect of temperature of chlorine solution (100 mg/l, pH 6.0) on inactivation of E. coli inoculated on broccoli florets

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Mean log_{10} CFU/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>3.85</td>
</tr>
<tr>
<td>8</td>
<td>3.71</td>
</tr>
<tr>
<td>15</td>
<td>3.92</td>
</tr>
<tr>
<td>20</td>
<td>3.94</td>
</tr>
<tr>
<td>25</td>
<td>3.93</td>
</tr>
<tr>
<td>No dip</td>
<td>6.19</td>
</tr>
<tr>
<td>LSD (P = 0.05)</td>
<td>0.49</td>
</tr>
</tbody>
</table>

*Broccoli was dipped for 2 min. Concentration of inoculating suspension: 7.16 log_{10} CFU/ml. E. coli were not detected on uninoculated, undipped broccoli.

was no significant linear effect of contact time for the water dip and the 50 ppm chlorine dip. In the 100 ppm chlorine treatment there was a significant linear decrease as time was increased. When broccoli florets were inoculated with a suspension containing 2.62 log_{10} CFU/ml the numbers were reduced to the extent that only seven experimental units had cells detected (data not shown). E. coli were not detected on uninoculated, undipped broccoli.

3.3. Effect of temperature of chlorine on inactivation of E. coli inoculated on broccoli florets

The population of E. coli found on the broccoli following inoculation was 1 log_{10} count lower than that in the suspension (Table 3). Dipping the broccoli for 2 min in 100 mg/l chlorine solution significantly reduced E. coli cells by between 2.3 and 2.5 log_{10} CFU/g compared with inoculated undipped broccoli (Table 3). This is comparable with the reduction seen in the previous broccoli dipping experiment for 100 mg/l chlorine and 2 min dipping duration. No significant effect of dip temperature was observed. E. coli were not detected on uninoculated, undipped broccoli.

4. Discussion

Chlorine was not found to be particularly effective at eliminating E. coli cells inoculated onto broccoli or lettuce. Reductions of 1.7–2.8 log_{10} CFU/g were observed when broccoli or lettuce were inoculated with high concentrations of E. coli. However, water alone reduced numbers by 1.5–1.8 log_{10} CFU/g. This is comparable with results observed by Brackett (1987) who found that dipping Brussels sprouts in 200 mg/l chlorine for 10 s reduced L. monocytogenes concentration by 2 log_{10} CFU/g and dipping in water reduced counts by 1 log_{10} CFU/g. It should be considered that the reductions seen in these experiments were under ideal conditions, where for each sample a fresh dipping solution was prepared using deionised water and the pH was optimally adjusted to maximise the hypochlorous acid concentration. Furthermore, the inoculum and dipping method employed represents recent contamination and would not allow for adherence of the E. coli to the produce surface or to biofilms. Consequently, the reduction in viable E. coli cell numbers observed here may be higher than those achieved under commercial conditions.

Differences in the level of E. coli deactivation were observed between lettuce and broccoli. For lettuce, counts were significantly reduced by exposure to 50 mg/l chlorine for 30 s compared with water. Longer exposure times or a higher concentration of chlorine did not have any significant additional benefit. Whereas for broccoli, a contact time × concentration interaction was found and in some combinations water was as effective as chlorine. This may be due to the difference in morphology. Broccoli is protected by a relatively thick waxy cuticle that repels water and possibly discourages bacteria from adhering to its surface. This may also prevent biofilm formation that has been observed in leafy vegetables (Morris and Nguyen-The, 1996). Biofilms are known to offer some protection to microorganisms within the biofilm. However, Seo and Frank (1999) found that E. coli 0157:H7 did not preferentially adhere to bioform produced by Pseudomonas fluorescens on the leaf surface of lettuce. It is unlikely that the differences observed were because of differences in chlorine demand due to organic matter in the dipping solution, as for both lettuce and broccoli, the volume of dip compared with the amount of plant material was very large.
No significant differences were observed in the level of cell inactivation during a 2 min dip in 100 mg/l chlorine solution at temperatures between 4 and 25°C. Chlorine efficiency in vitro increases with temperature up to the point before vaporisation occurs (Boyette et al., 1993). Thus, in vivo effects may differ from those in vitro. Conversely, El-Kest and Marth (1988) found that chlorine was more effective in killing L. monocytogenes at 5°C than at 25 or 35°C. Hoffman et al. (1981) found that lowering the pH of the chlorine solutions resulted in less stability of the available chlorine at 25°C. In a study looking at the effect of temperature on coliform and enteric pathogens, 25°C was more effective than 5°C and the greatest differences occurred at pH values higher than 8.5 and at low concentrations of chlorine (El-Kest and Marth, 1988). It would seem that the effectiveness of chlorine in killing pathogens at different temperatures is complex and involves a number of factors.

These results show that chlorine reduces E. coli populations on produce surfaces. However, it cannot be assumed that it will completely eliminate pathogens. Chlorine washing should be used to complement a production system that maintains cleanliness at all stages of production and postharvest handling. It can not be relied on as a treatment to ensure produce is free of bacteria which may be harmful to humans if consumed on raw produce.

Acknowledgements

The work presented in this paper was supported by the Horticultural Research and Development Corporation (project number VG 98093) and the DNRE.

References