Minimally processed vegetable products generally require no further treatment and are eaten raw, posing a potential contamination of food pathogens. Outbreaks of *Escherichia coli* 0157: H7 (EHEC) have been linked to fresh produces such as alfalfa sprout, radish sprout, and lettuce\(^2\). EHEC infection could lead to kidney complications, hemolytic-uremic syndrome and possibly death\(^3\). Fresh leafy produce has carried the 10 organisms needed for illness therefore understanding the behavior of EHEC on produce is essential.

This pathogen can grow from around 70\(^\circ\)C to 100\(^\circ\)C, with an optimum temperature of 37\(^\circ\)C. Adjusting the storage temperature to 4\(^\circ\)C promotes stress condition to the bacteria. Washing reduces the total microbial load, prevents spoilage, and minimally processed vegetable products generally require no further treatment and are eaten raw, posing a potential contamination of food pathogens. Outbreaks of *Escherichia coli* 0157: H7 (EHEC) have been linked to fresh produces such as alfalfa sprout, radish sprout, and lettuce\(^2\). EHEC infection could lead to kidney complications, hemolytic-uremic syndrome and possibly death\(^3\). Fresh leafy produce has carried the 10 organisms needed for illness therefore understanding the behavior of EHEC on produce is essential.

The hypothesis is that EHEC on lettuce leaves over time:
- Refrigeration (4\(^\circ\)C) slows adherence of EHEC to lettuce.
- Increased incubation time will lead to increased adherence of EHEC on lettuce.
- Chlorine is the most effective washing solution to remove EHEC on lettuce leaves.

### Methods

Adherence of EHEC on lettuce leaves over time:
- Incubate EHEC culture at 37\(^\circ\)C for 24 hours.
- Correlate concentration (CFU/ml) with absorbance.
- Cut pieces of 3x3cm romaine lettuce, record each weight, and wash with distilled water.
- Inoculate pieces of lettuce with 10\(^6\) CFU/0.1ml EHEC, place three lettuce on petri dishes to store in fridge (4\(^\circ\)C) for 0, 1, 2, 4, 6 hours and room temperature (20\(^\circ\)C) for 0, 6, 24, 48 hours.
- Rinse with distilled water to dislodge bacteria.
- Blend in 90 ml Phosphate Buffer Saline (PBS) for 30 seconds with stomacher.
- Serially dilute and plate on Eosin-methylene blue (EMB). However, by 6 hour many more bacteria had adhered to the lettuce than by 48 hour at 4\(^\circ\)C. This supports the hypothesis that EHEC does not adhere as well to lettuce at colder temperatures. Further study needed to determine if only more EHEC are adhering or if the increase is due to replication.
- To determine if EHEC is removable from lettuce by chlorine or alcohol washes, Lettuce treated with EHEC for 6 hour was washed for 5 minutes with the different solutions. 5 minutes contact time with 1% chlorinated water could eliminate EHEC detectable levels of EHEC. Alcohol did not remove the bacteria from the lettuce.

The results indicate that chlorine washes and storage at 4\(^\circ\)C would minimize ability of EHEC to remain on lettuce.

### Results

Table 1. The % Survivor of EHEC after soaked in chlorinated wash solution

<table>
<thead>
<tr>
<th>Washing solution</th>
<th>% Survivor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled H(_2)O</td>
<td>18.30</td>
</tr>
<tr>
<td>0.1% Cl(_2)</td>
<td>6.26</td>
</tr>
<tr>
<td>1% Cl(_2)</td>
<td>0.00</td>
</tr>
<tr>
<td>0.1% Alcohol</td>
<td>18.20</td>
</tr>
<tr>
<td>1% Alcohol</td>
<td>17.95</td>
</tr>
</tbody>
</table>

Concentration less than 10 CFU/ml could not be detected

### References


### Acknowledgements

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