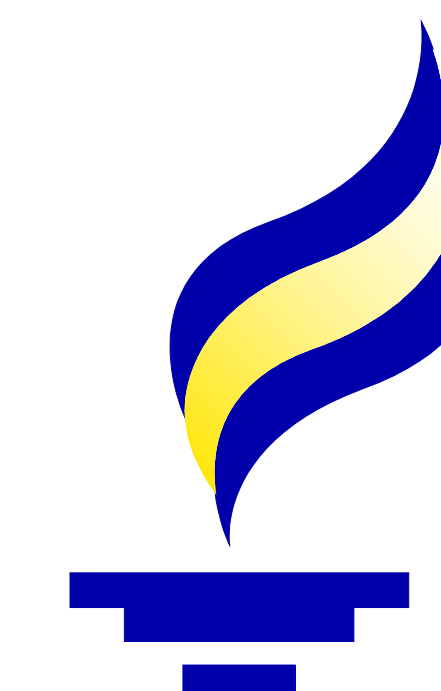


The Adherence of Escherichia coli 0157:H7 on Lettuce Leaves over Time And Removal Technique using Chlorinated water

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Background

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 produce such as alfalfa sprout, radish sprout, and lettuce^{2,4}. EHEC infection could lead to kidney complications, hemolytic-uremic syndrome and possibly death³. 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 7⁰-10⁰C to 50⁰C, with an optimum temperature of 37⁰C. Adjusting the storage temperature to 4⁰C promotes stress condition to the bacteria. Washing reduces the total microbial load, prevents spoilage, and maintains quality¹. Sodium hypochlorite (Bleach) is a chlorine based chemical that is permitted to use as washing agent for sanitizing vegetables for food manufacture¹. The purpose of this research is to compare the adherence of EHEC on lettuce leaf stored in fridge (4⁰C) and room temperature (20⁰C) for different time intervals. Several wash solutions (chlorinated water, distilled water, alcohol solutions) are being used to determine the effectiveness of removing EHEC on lettuce leaves.

Hypothesis

- Refrigeration (4⁰C) slows adherence of EHEC to lettuce
- Increased Incubation time will lead to increased adherence of EHEC on lettuce leaves
- Chlorine is the most effective washing solution to remove EHEC on leaves compared to distilled water and alcohol wash solution

Methods

Adherence of EHEC on lettuce leaves over time:

- Incubate EHEC culture at 37⁰C 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 10⁵ CFU/0.1ml EHEC, place three lettuces on petri dishes to store in fridge (4⁰C) for 0,1,2,4,6 hours and room temperature (20⁰C) 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)

Removal Technique with different wash solutions:

- Inoculate pieces of lettuce with 10⁵ CFU/0.1ml EHEC
- Incubate at room temperature (20⁰C) for 6 hours
- Fill a petri dish with 20 ml of wash solution (chlorinated water, distilled water, or alcohol solutions)
- Place the three contaminated lettuces to each petri dish, leave to soak for 5 minutes
- Blend in 90 ml Phosphate Buffer Saline (PBS) for 30 seconds with stomacher
- Serially dilute and plate on Eosin-methylene blue (EMB)

Results

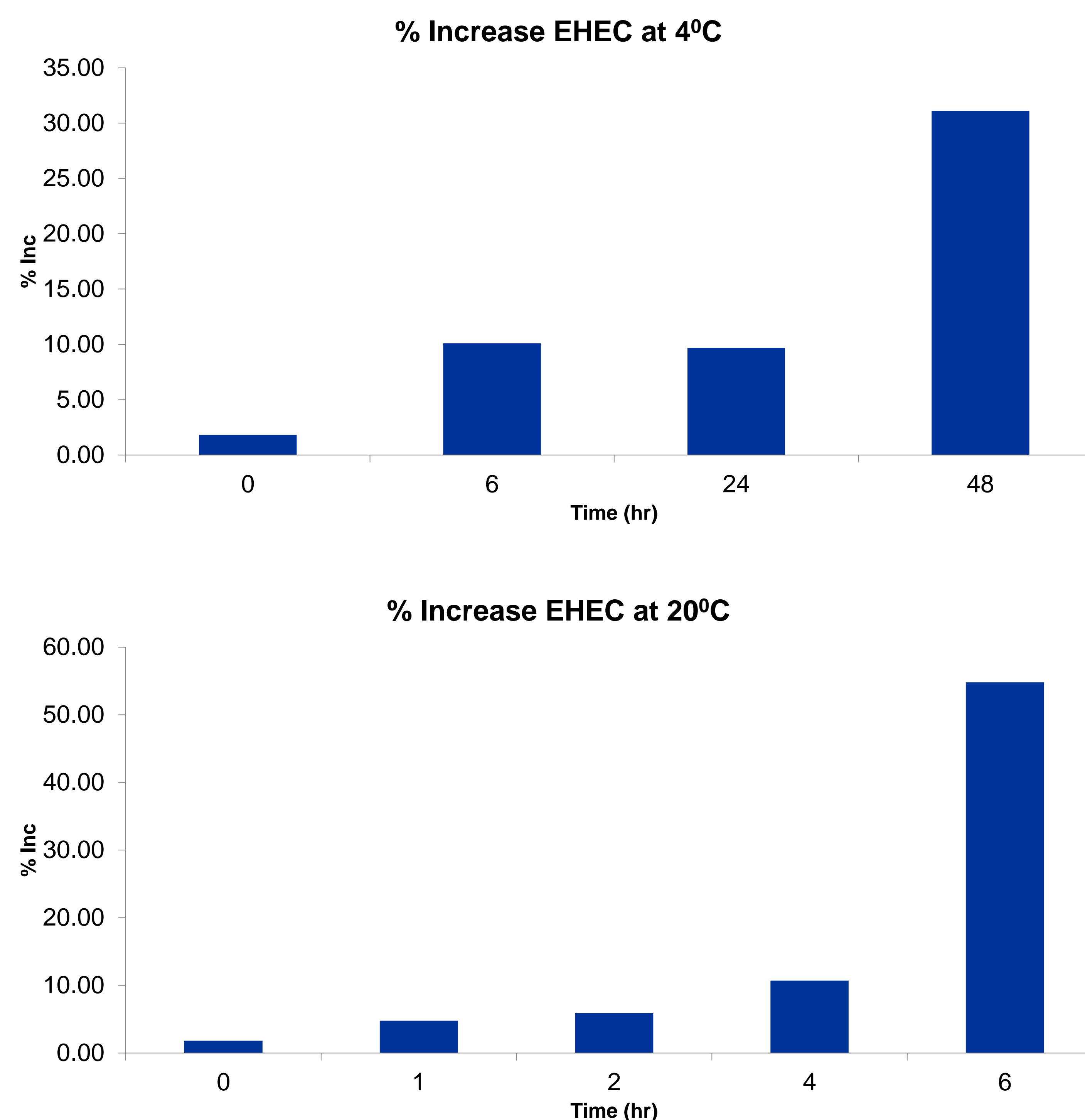


Table 1. The % survivor of EHEC after soaked in chlorinated wash solution

Washing solution	% Survivor
Distilled H ₂ O	18.30
0.1% Cl	6.26
1% Cl	0.00
0.1% Alcohol	18.20
1% Alcohol	17.95

* Concentration less than 10 CFU/g could not be detected

Conclusion

- Low numbers of EHEC bind rapidly to lettuce. At 4⁰C, 10% of the inoculated bacteria had adhered to the lettuce by 6 hour. The number of adhered bacteria did not change significantly by 24 hour. The number increased greatly by 48 hour. At 20⁰C, sampling times were shortened because *E. coli* replicates at 20⁰C. The pattern of adhered bacteria was similar to the refrigerated sample. By 1 and 2 hour the number of adhered bacteria was greater than the initial sample, but not significantly different from the 4⁰C samples of 6 and 24 hour. By 48 hours many more bacteria were adhered to the lettuce. Since EHEC doesn't replicate at 4⁰C well, this numerical increase is more likely due to adherence of the inoculated bacteria than to growth. By 6 hour at 20⁰C, EHEC is replicating slowly. Therefore, the increase in number at 6 hour could be due to either replication of the adhered bacteria or to adherence of more bacteria from the initial inoculum. However, by 6 hour many more bacteria had adhered to the lettuce than by 48 hour at 4⁰C. 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 4⁰C would minimize ability of EHEC to remain on lettuce.

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