Effects of pH on *E. coli* K12 in Spinach

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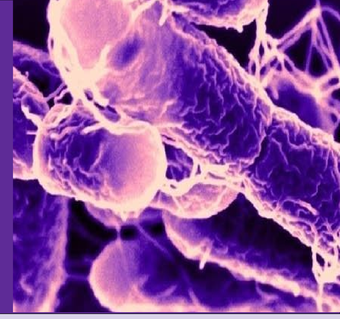
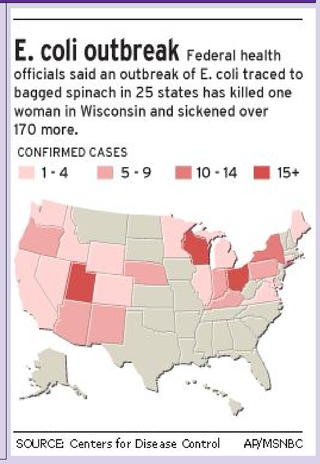


Introduction

- *Escherichia coli* is one of the more well-known foodborne pathogens. While it can be found in the intestines of most animals, *E. coli* can also infect a number of foods, including raw meats and produce (CDC, 2015).
- There has been concern with *E. coli* on green, leafy vegetables due to their large surface area(Wang et. al, 2010). Yet, due to data from *E. coli* outbreaks in 2006, this suggests that the bacterium may be internalized in leaves(Wang et. al, 2010). While not pathogenic, *E. coli* K12 will be used as the experimental bacterium for this study .



Objectives

- Determine presence of *E. coli* K12 on bagged spinach and compare to a negative control.
- A treatment was added to the positive controls of lemon juice that has a pH level of 2 and 4 and measured how much it helps inhibit growth of *E. coli* K12

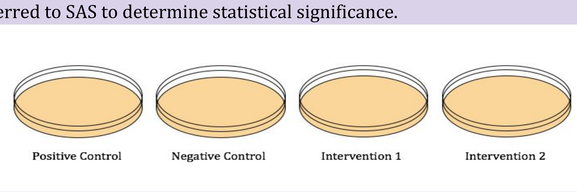
Materials and Methods

Inoculation
Conventionally produced, triple washed RTE spinach was spotted with 100 µL of *E. coli* K12. The culture was allowed to adhere for 30 minutes for maximum cell attachment.

Intervention preparation
Bottled lemon juice was tested with litmus paper to confirm its pH of 2 for Intervention 1. The juice was then diluted with DI water to a pH of 4 to create Intervention 2. 5 mls of each preparation were applied and let sit for 5 minutes.

Plating
PCA, PDA, and McConkey plates were plated with all 4 treatments, using dilutions of 10^{-3}, 10^{-4}, and 10^{-5} on days 0 and 2. On day 7, intervention 1 was plated using 10^{-1}, 10^{-2}, and 10^{-3} dilutions. Furthermore, no negative controls were plated on day 7.

Enumeration
All plates were counted manually and data recorded in Excel. Excel data was then transferred to SAS to determine statistical significance.



Results

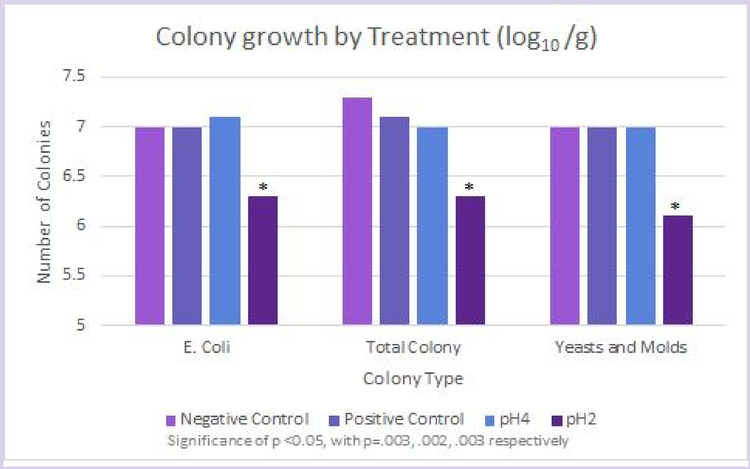


Figure 1

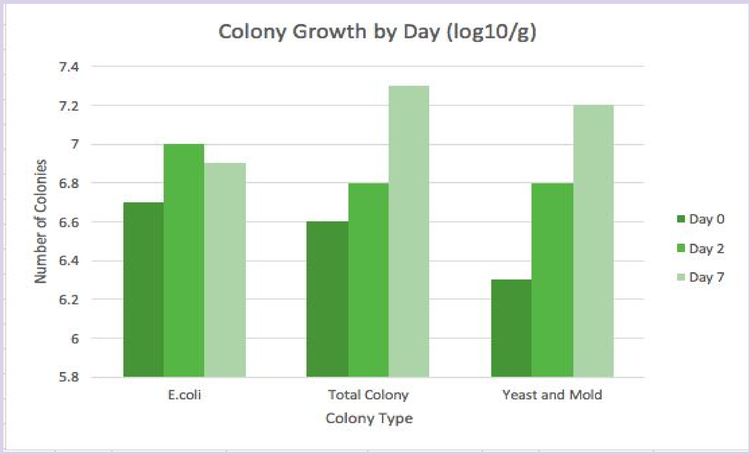


Figure 2

Impact of pH on E.coli K12 in spinach, reported in $\log_{10}$/g.

	Treatment					Day				*P* =		
Item;	Negative Control	Positive Control	pH4	pH2	Pooled SEM	0	2	7	Pooled SEM	Treatment	Day	Treatment × Day
E. coli	7.0^{a}	7.0^{a}	7.1^{a}	6.3^{b}	0.16	6.7	7.0	6.9	0.15	0.003	0.353	0.400
Total Colony	7.3^{a}	7.1^{a}	7.0^{a}	6.3^{b}	0.17	6.6^{b}	6.8^{b}	7.3^{a}	0.15	0.002	0.005	0.125
Yeast and mold	7.0^{a}	7.0^{a}	7.0^{a}	6.1^{b}	0.17	6.3^{c}	6.8^{b}	7.2^{a}	0.15	0.003	0.001	0.162

^{abc}Means within a row that do not share a common superscript differ $P < 0.05$.

Figure 3

After plate counts, the negative control, positive control, and pH 4 treatment all had similar results for growth of *E. coli*, yeasts and molds and total growth. The pH 2 treatment had a statistically significant decrease across all plates and without alteration from general day-to-day growth. The statistical significance of the treatment was .003 for *E. coli*, .002 for Total Colony, and .003 for Yeasts and Molds

Significance

The treatment of undiluted lemon juice is a viable control method not only in regard to *E. coli* but also for other bacterial, yeast, and mold growths.

While pH has been considered in *E. coli* control, rarely has an appropriate substance been used that would be consumable with fresh produce such as spinach. The possibility of small quantities of lemon juice being used as a bacterial control can be applied in situations where spinach is left out over long periods of time or in areas with little environmental control.

There are several different directions the research can take from here. The exact pH for maximum growth inhibition could be somewhere in between our treatments. Specific types of spinach could also be checked.

References

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http://media4.s-nbcnews.com/i/msnbc/Components/Interactives/Health/MiscHealth/E_Coli_outbreak_925.gif

Acknowledgements

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