FINAL RESEARCH REPORT
Test of Alka-Hydroxy Antibacterial Effect on Spinach Leaves
December 4, 2006

Purpose

The purpose of this experiment is to test the antibacterial effect of Alka-Hydroxy product on spinach leaves inoculated with *Escherichia coli* O157:H7.

Procedure

1. Ten grams of fresh spinach leaves are weighed out on a top-loading balance, and the leaves are then transferred into a clean, plastic (24 oz.) food storage container. Three separate samples are prepared (30 g total).

2. Using sterile forceps, the leaves are dipped into a 250-ml solution containing a 1:200 dilution of an overnight culture of *E. coli* O157:H7 cells in sterile phosphate-buffered saline (PBS), pH 7.0 (1×10^7 cfu per ml) and soaked for 1 min. The inoculated leaves are then returned to the plastic containers and drained of excess liquid for 10 min.

3. Two and five percent solutions of Alka-Hydroxy (250 ml each) are prepared by diluting the concentrate in sterile distilled water.

4. The leaves are transferred to clean plastic containers to soak in the following solutions with gentle agitation: sterile distilled water, 2% Alka-Hydroxy, and 5% Alka-Hydroxy. The leaves are removed after 10 min and allowed to drain of excess liquid for 10 min.

5. The leaves from each treatment are transferred to a sterile Waring blender with 190 ml of sterile PBS, pH 7.0 and blend for 1 min at the low-speed setting. This homogenized sample is considered to be a 5×10^{-2} dilution.

6. Each homogenized sample is further diluted in sterile PBS. The additional dilution series is 1×10^{-2} (1.0 ml:4.0 ml), 1×10^{-3} (1.0 ml:9.0 ml), and 1×10^{-4} (1.0 ml:9.0 ml). Samples (0.1 ml) are inoculated from each dilution onto Luria agar plates (nonselective medium) and sorbitol MacConkey agar plates (selective medium) in triplicate using the spread-plating technique. All of the plates are incubated for 24–48 hours at 37°C.

7. The number of colonies on each plate are counted, and the colony-forming units per ml (cfu/ml) are calculated for each of the treatment conditions.

Results

	Sorbitol MacConkey Agar* 1 x 10^{-4} dilution			Luria Agar 1 x 10^{-5} dilution		
No ERA:	348	169	190	351	308	295
	avg = 235.7 std. dev. = 97.849 **avg cfu/ml = 2.36 x 10^6**			avg = 318.0 std. dev. = 29.309 **avg cfu/ml = 3.18 x 10^7**		
2% ERA:	42	34	43	90	103	104
	avg = 39.7 std. dev. = 4.933 **avg cfu/ml = 3.97 x 10^5**			avg = 99.0 std. dev. = 7.810 **avg cfu/ml = 9.90 x 10^6**		
5% ERA:	30	22	32	102	103	97
	avg = 28.0 std. dev. = 5.292 **avg cfu/ml = 2.80 x 10^5**			avg = 100.7 std. dev. = 3.215 **avg cfu/ml = 1.01 x 10^6**		

* Counts of sorbitol-positive (red) colonies.

Conclusions

Total colony counts were reduced by 69% and *E. coli* O157:H7 counts by 83% when treated with 2% Alka-Hydroxy product compared to the control (treatment with sterile distilled water). Treatment with 5% Alka-Hydroxy had no additional effect on total counts and only a marginal effect (reduction to 88%) on the *E. coli* O157:H7 counts. Thus, the 5% concentration does not appear to confer any significant added benefit over the 2% concentration.

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