# Efficacy of Household Agents in the Removal of *E. coli* from Produce


## Abstract

*Common household chemicals may provide a safe an inexpensive option for use as agents to wash fresh produce and reduce the population of foodborne pathogens such as E. coli. A comparison of the efficacy of two household agents on the reduction in E. coli population is reported here. A 0.01% concentration of bleach solution and a 5.00% acetic acid solution were used as washing agents on spinach and tomatoes that had been inoculated with E. coli. Comparisons of total E. coli and total aerobic bacteria following various washing techniques were made. Spinach treated with bleach or acetic acid produced little to no reduction of total bacteria in the population. Tomatoes treated with acetic acid showed a 30-95% reduction in total aerobic bacteria, while tomatoes treated with bleach showed no growth of bacteria following a 48 hour incubation period. Future work will investigate the use of a genetically modified E. coli isolate as an inoculum source to more effectively and efficiently distinguish E. coli from total aerobic bacteria.*

Most strains of the bacteria *Escherichia coli* are harmless. Some strains can make humans very sick, causing a wide array of symptoms such as urinary tract infection, respiratory illness, diarrhea and others. *E. coli* causes disease in humans by making toxins, referred to as shiga toxins (or STEC); the most common and most frequently reported in the media is *E. coli* O157:H7 ("*Escherichia coli*," 2008). The negative effects of this strain contribute to recent consumer insecurities to purchase fresh produce such as spinach. According to the center for disease control and prevention (CDC), the *E. coli* outbreak of 2006 affected 26 states with reports of 95 people hospitalized, 29 developing hemolytic uremic syndrome (HUS) and at least one death. The sources of spinach came from Utah, New Mexico and Pennsylvania (*MMRW*, 2006). The effects of *E. coli* outbreaks are of serious concern for individuals with compromised immune systems such as children, the elderly and pregnant women.

Although eliminating or reducing fresh produce from the Western diet would reduce the incidence of *E. coli* infection, it would be detrimental to the health of the population due to factors such as nutrient loss during food processing (Schroeder, 1971). Fresh produce also provides distinct flavor, texture, crispness, and mouth feel unique to a given food item. Cooking, canning, and other methods of processing eliminate a large majority of foodborne pathogens, but only to sacrifice taste, texture, nutrition and other properties of food. Therefore, a need persists in the fresh-food industry for practices beyond standard water rinsing, to safeguard the fresh produce industry. Economical value is also a growing concern for washing technique. Fit™ spray is a product available in supermarkets and contains a variety of ingredients including citric acid, baking soda, ethyl alcohol, oleic acid, and glycerol. A gallon of that product will cost $30 or more, with a per-use of a few milliliters for spraying produce to ½ cup per gallon of water for soaking methods (Fit FAQs, 2009). A gallon of bleach can cost between two and three dollars, and a gallon of 5% acetic acid, or vinegar, costs about five dollars. Bleach, the most economical of the three, is further diluted when used directly on food. According to federal regulations, utilizing household bleach at a concentration of 100 parts per million (0.01%), or approximately one tablespoon per gallon of water, on food and food surfaces is the maximum amount recommended (McGlynn, n.d.). Contact with food or other surface is recommended for one to five minutes to ensure sufficient sterilization (McGlynn, n.d.).

Acetic acid is used in products such as pickles and ketchup to prevent bacterial growth from occurring (Dauthy, 1995). Dr. Carol Seaborn (personal communication November 12, 2008) emphasized research in a controlled laboratory study reporting more effective anti-microbial results from using organic acid treatment on *Salmonella enterica* combined with additional water rinses, drying, and agitation procedures on apples, versus water washing alone.

The objective of the present research was to determine the effectiveness of the common household chemicals bleach and vinegar as washing agents to remove bacteria from inoculated tomatoes and spinach. It was hypothesized that using either acetic acid or bleach solutions in place of water alone will provide sufficient anti-microbial protection and eliminate growth of *E. coli* and other harmful bacteria.

## Method

### *Materials*

<u>Fresh Produce Sources:</u> Commercially available washed and bagged spinach leaves and boxed grape tomatoes were purchased at a local grocer and refrigerated at 4°C for 12 hours before use.

<u>E. coli Inoculum:</u> Sterile 3ml culture tubes of nutrient broth media were inoculated with ~10µl of *E. coli and* were incubated overnight at 37°C. McFarland standards were used to

visually approximate the concentration of cells in suspension. The McFarland scale represents the specific concentrations of Colony Forming Units (CFU); the amounts used in ratio to make a 0.5 standard are Barium Chloride ($BaCl_2$) to Sulfuric Acid ($H_2SO_4$) is .05 to 9.95 (Sutton, 2006). Once the *E. coli* mixture was consistent with the McFarland standard, the cell density approximated $10^6$ CFU/mL.

Media: Samples were cultured on Luria Agar with and without ampicillin.

*Design*

This research was executed by students in an undergraduate microbiology class at the University of Wisconsin-Stout, who performed the *E. coli* inoculation of the produce and washing with appropriate chemical agent. Two class sections were assigned to different washing techniques (either bleach or acetic acid) and different fresh produce (either tomato or spinach). Both uninoculated (control) and inoculated (experimental) controls were included in the study.

*Procedure*

Samples were inoculated to establish consistent inoculum to produce ratios. For spinach, 0.5 g of fresh produce was used with 500 µL of *E. coli* inoculum. For the tomato, 10 g of fresh produce was inoculated with 1mL of *E. coli*. For the both the spinach and tomato control groups, 1 mL of sterile distilled water was used per 10 g of sample. Weighed produce samples were placed in sterile stomacher bags and inoculated with either *E. coli* (experimental) or water (control). Following inoculation, the bags were placed on a hard surface in an upright position for 20 minutes. After the adhering time, 10 mL of assigned wash solution (either acetic acid or bleach) was added to each bag. Solution was gently rubbed, decanted, and rinsed three times for 30 seconds each time, with 10 mL quantities of sterile water.

Following the rinsing procedure, each sample was placed into separate, sterile stomacher bags. After adding 10 mL of sterile water to each of the samples, bags were manually agitated and rubbed thoroughly, without tearing or puncturing the external surface of the sample. After 10 minutes of agitation, 1 mL of sample was removed from each stomacher bag with a pipette, directly onto separate lb/amp plates. The solution was spread with a sterile bent glass rod. Plates were labeled and placed for 48 hours into a 37°C incubator.

## Results

Bacterial growth was measured in values of 0-10; 0 representing no growth on the agar plate and 10 representing 100% plate growth coverage. The serial dilution controls, with the uninoculated sample (taken directly from the ready-to eat package), covered 50% of the plate surface from the tomato, and 80% growth from the spinach. The inoculated samples for both the tomato and spinach each had 80% growth using the sterile water serial dilution method.

One spinach group receiving the acetic acid treatment had 100% growth in both the control and treatment groups. The second spinach group had 70% growth on the uninoculated plate and 90% growth on the plate inoculated with *E. coli*.

One bleach treatment group showed 100% growth on the both the inoculated and uninoculated plates; the second bleach treatment group covered 90% of the uninoculated plate and 100% coverage on the plate inoculated with *E. coli*.

The first control group, which used the tomato treated with acetic acid, produced 50% growth on the uninoculated plate and 70% growth on the inoculated plate. The second treatment group covered 5% of both the uninoculated and inoculated plates.

Both tomato bleach treatment groups showed no growth for both inoculated and uninoculated plates. See Tables 1 through 3 for condensed descriptions of the results.

**Table 1**

*General Bacterial Growth on Agar Plates for Two Washing Treatments—Class Results*

| Tomato—Acetic Acid | Tomato—Bleach |
| --- | --- |
| Group 1 | Group 1 |
| Uninoculated 50% | Uninoculated 0% |
| Inoculated 70% | Inoculated 0% |
| Group 2 | Group 2 |
| Uninoculated 5% | Uninoculated 5% |
| Inoculated 5% | Inoculated 0% |

**Table 2**

*General Bacterial Growth on Agar Plates for Serial Dilution Control—Class Results*

| Spinach—Acetic Acid | Spinach—Bleach |
| --- | --- |
| Group 1 | Group 1 |
| Uninoculated 100% | Uninoculated 100% |
| Inoculated 100% | Inoculated 100% |
| Group 2 | Group 2 |
| Uninoculated 70% | Uninoculated 90% |
| Inoculated 90% | Inoculated 100% |

**Table 3**

*General Bacterial Growth—Authors' Results*

| Spinach | Tomato |
| --- | --- |
| Uninoculated 80% | Uninoculated 50% |
| Inoculated 80% | Inoculated 80% |

## Discussion

The growth percentages represent *E. coli* and other bacterial growth; due to the tremendous amount of variable bacterial colonies, all growth was included in the study. Due to insufficient time, gram stains were unable to be performed on individual colonies. Future research will focus on the use of genetically modified *E. coli* that is more easily distinguishable from the general bacterial population.

Both the bleach and acetic acid treatment groups for the spinach had greater rates of general bacterial growth on the plates inoculated with *E. coli* than the plates not inoculated. This result was expected due to the addition of bacteria. In the Tomato groups, however, only one acetic acid group showed this relationship, while the other groups had 5% or less growth on inoculated and uninoculated plates, with the bleach group completely clear of any colony growth. These results support the hypothesis that bleach and acetic acid treatments function as anti-microbial agents; however, the inconsistencies between the tomato and spinach groups leave room for speculation on possible differences between spinach and tomatoes. In the study performed on the *E. coli* outbreaks of Utah and New Mexico in 2006, Grant et al. (2008) explained that *E. coli* adherence to spinach surfaces can occur by internalization of the plant structure by entrance through the roots of the plant and adherence is more likely to occur on cut surfaces, such as pre-packaged containers. Therefore, excessive bacterial growth on the spinach can be explained by textural and structural differences between the produce.

This study may have benefitted from a third group measure of water-only rinsing. Including this step could explain the discrepancy between the inoculated and uninoculated groups. Vegetables in pre-packaged containers are assumed “ready to eat,” and an additional water rinsing step may have produced similar effects as the wash treatments. Therefore, by incorporating another group including a comparison to water wash could provide a more thorough comparison of traditional household washing.

An interesting finding in this study was the similarity of colony growth on the inoculated and uninoculated plates for spinach with both washing treatments. It is possible that bacterial growth increases from food taken directly out of the package and eaten without any washing. In other words, high levels of bacterial growth already present on the produce may have interfered with additionally added *E. coli,* and may even promote the bacterial growth population upon inoculation. It is possible that the impact of the treatment was actually reduced, but due to the high amount of bacteria already present on the spinach, no effects were detectable from the treatment.

Using tomatoes in the study of *E. coli* growth may also have an impact on the results of this study. Historically, tomatoes have been infected with *Salmonella* rather than *E. coli*. This factor may be related to some individual differences between the two vegetables. Further research could investigate the impact of *Salmonella* inoculations with each of the washing treatments, to see if the treatments have the same effectiveness on a more “predisposed” produce. Another interesting component regarding the contamination of tomatoes is the link primarily to recurrent outbreaks of the same growing regions (*MMWR Weekly*, 2007).

Further research could focus specifically on *E. coli* growth by using transformed bacteria and ampicillin as a selective media. This media should also contain the sugar arabinose, which causes transformed bacteria to glow under UV light. This mechanism is enabled by the Green Fluorescent Protein (GFP) gene (“PGlo transformation,” n. d.). By placing the inoculated plate under ultraviolet light, a researcher can easily isolate the growth of the *E. coli* inoculated in the sample regardless of bacterial growth amount. This method could also aid in measuring the fastidiousness of *E. coli* on different types of produce.

There are many factors which can contribute to the effectiveness of antimicrobial agents: population size—larger populations are more persistent than smaller ones, the presence of resistant endospores, the concentration of the agent, duration of exposure to agent, temperature, environment, biofilm production, and heat and acidity. It is also important to consider the type of pathogen the anti-microbial agent is being used for. Bacteria have different adaptation capabilities which impact the ease of eliminating the pathogen.

In the United States, the increasing number of outbreaks of pathogenic infections poses concern for food safety, and illustrates the need for safe, effective, and inexpensive washing techniques to prevent human infection during consumption of fresh produce. Current regulations of use for household bleach as an antimicrobial agent have been shown to be safe and effective when standards are followed; but perhaps society is in need of more universal practice to prevent infection outbreaks, and to assume all produce contains pathogenic microorganisms. Future research could further define factors enabling pathogenic spread and control, and determine other inexpensive means of effectively eliminating bacterial growth on produce.

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