EFFICACY COMPARISON OF FIVE CLEANING SOLUTIONS IN DECONTAMINATION OF ESCHERICHIA COLI IN LETTUCE.

Panchalee Pathanibul* **& Choosit Hongkulsup****
*, ** *Faculty of Science and Technology, Suan Sunandha Rajabhat University, Bangkok, Thailand*
*E-Mail: *panchalee.pa@ssru.ac.th, **choosit.ho@ssru.ac.th*

ABSTRACT

Vegetables are healthy additions to human diets nowadays. They are excellent sources of vitamins and minerals that are essential for many functions in the body. However, fresh vegetables are frequently contaminated with foodborne pathogens. Vegetable washing is an important step to help minimize the amount of harmful bacteria prior to consumption.

Efficacy of five washing solutions in reducing *Escherichia coli* contamination in lettuce was investigated in the present study. Three tested solutions were derived from household ingredients consisting of lime, salt and vinegar. One washing solution was obtained from a local herb, Gotu kola, recently reported to exhibit antimicrobial property. The last washing solution prepared from potassium permanganate, a chemical long used in vegetable cleaning, was also compared.

Lettuce was artificially inoculated with *E. coli* of about 8 log CFU/g. Following soaking in tested solutions for as long as 30 min, 15% lime solution was shown to be the most effective solution in disrupting *E. coli* cells achieving a 1.53-log reduction. The rest of the solutions in order of antibacterial effectiveness, 200 ppm potassium permanganate, 10% vinegar, 20% Gotu kola and 1.5% salt, were only able to destroy little amount of *E. coli* (less than 1 log CFU/g in all treatments). Results provided preliminary data and indicated the feasibility for further improvement of natural ingredient application in vegetable washing.

Keywords: *Escherichia coli*, Gotu kola, lettuce, lime, potassium permanganate, salt, vinegar

INTRODUCTION

Vegetables are categorized as one of five main food groups for human consumption. It is recommended that vegetables should be included in daily food intake because they are abundant in vitamins, minerals, dietary fibers and phytochemicals. These essential nutrients assist in proper functions of the body and provide health benefits. As the healthy eating trend is on the rise, more vegetables are consumed globally. Fresh vegetables are preferred over cooked ones owing to the greater quantity of nutrients. Salad is the most popular form of dishes with fresh vegetables. In Thailand, many vegetables are consumed fresh by dipping them in various kinds of spicy sauce as side dishes. This consumer behavior, however, raises a safety concern of foodborne illnesses associated with potential pathogen contamination in vegetables. Crops grown in farms are naturally contaminated with microorganisms. Utilization of animal manure can also transfer unwanted microbes to produce. Despite quality control measures, *Salmonella* spp. and *Escherichia coli* have been consistently reported to tarnish crops exported to Europe [1]. It is likely that farm grown produce sold in local markets without good agricultural practices harbors even higher microbial counts. If vegetable preparation or washing is not performed appropriately before eating raw, consumers will be at an increased risk of getting sick from exposure to high microbial loads and even toxins derived from the microorganisms. In a microbiological safety survey of foods sold in one university in Thailand, over 38% of pre-cooked food samples collected from cafeteria and kiosks was reported to be contaminated with coliform bacteria [2]. Therefore, an extra caution should be taken for vegetables preferably eaten fresh.

In order to diminish microbial contamination, chemical solutions have been employed to clean fresh vegetables. Vegetable washing is the only step to help decrease the contaminants in vegetables consumed raw. Chemical compounds approved by the United States Food and Drug Administration (USFDA) to sanitize food surfaces comprise chlorine, chlorine dioxide, iodophors and Quaternary Ammonium Compounds (QACs) [3]. Chlorine is widely used in the food industry. Nevertheless, vegetable washing using chlorine at home may not be practical. To maximize destruction of microorganisms, certain concentration and exposure length of chlorine are required making it quite difficult to prepare at home. Tap water containing low-level chlorine is simply used for household vegetable cleaning. However, efficiency of tap water in microbial removal cannot be assured due to its variable quality in different areas. In addition, sterile water was tested in washing lettuce contaminated with *E. coli* O157:H7. An approximate 1 log CFU/g was achieved after a 10-min wash [4].

In the current study, more convenient practices in vegetable washing for consumers at home were investigated. Four natural ingredients consisting of salt, vinegar, lime and a local herb, Gotu kola, were prepared as washing solutions and applied to fresh lettuce to test their efficacies in *E. coli* reduction. Moreover, potassium permanganate ($KMnO_4$), a chemical long used in cleaning produce, was examined to compare its antibacterial activity with the natural solutions. Salt, vinegar and lime are common household ingredients which are easy to find, cheaply priced and literally safe for consumers. Lime in particular is often included in a number of Thai popular dishes such as various kinds of curry, Pad Thai, Som Tum, and so on [5]. Most families have these ingredients already stocked up at home. It is thus convenient to use some of them for the purpose of vegetable washing to enhance microbiological safety. The herb plant, Gotu kola, is less common, but simply obtainable and inexpensive at local fresh markets. Gotu kola was chosen to engage in the study because it has recently been reported to exhibit an antimicrobial property [6]. Side vegetables such as cucumber and cabbage frequently used to decorate and co-consume with the main dishes were tested by washing with Gotu kola solution. Result revealed that 1.5 times more *E. coli* destruction was achieved in vegetables cleaned with Gotu kola solution than those cleaned with purified water. In our experiments, tap water was employed as a control variable to discriminate the bactericidal effect with other tested solutions. In ready-to-eat produce undergoing no heat treatment, the disinfection procedure prior to consumption is vital. *E. coli* is one of the pathogens causing a number of foodborne illnesses each year. This organism is likely to contaminate produce because it belongs to the group of fecal coliforms which is generally brought in through utilization of animal manure. The presence of *E. coli* could indicate the probable presence of other closely related pathogens such as *Salmonella* and *Klebsiella*. Some *E. coli* strains are especially harmful by producing toxins. Consequently, preventive measures should be put in place to obtain maximal elimination of this microbe.

OBJECTIVES

1. To compare efficacies of four natural detergents including salt, vinegar, lime and Gotu kola solutions as well as potassium permanganate in diminishing *E. coli* artificially contaminated in lettuce

2. To determine the most effective detergent concentration and length of detergent soaking time resulting in maximal reduction of *E. coli*

MATERIALS AND METHODS

1. Lettuce preparation

Lettuce was bought from local markets or supermarkets. A few outer leaves were removed as they contained lots of dirt and germs. Inner leaves were taken off the stalk one by one. The leaves were washed with tap water to get rid of any remaining dirt and soil. Then, they were washed two more times with sterile distilled water. Using flame-sterilized scissors, the leaves were cut into squares of 5 cm x 5 cm.

2. Washing solution preparation

3.2.1 Lime solution

Lime were bought from local markets. They were washed to remove dirt with sterile distilled water. Lime were cut into halves using flame-sterilized knife. Lime halves were then pressed by hands with gloves on to get the juice out. Then, the juice was diluted to 5, 10 and 15% v/v concentrations from the original juice using sterile distilled water.

3.2.2 Vinegar solution

A bottle of vinegar was bought from a supermarket. The vinegar was then diluted to 5, 7.5 and 10% v/v concentrations from the original vinegar using sterile distilled water.

3.2.3 Salt (saline) solution

Table salt was bought from a supermarket. It was then weighed out and dissolved in sterile distilled water to create 0.5, 1 and 1.5% w/v concentrations.

3.2.4 Gotu kola solution

Gotu kola leaves were bought from local markets. 200 g of the leaves were boiled in 500 ml of water. Then, the wetted leaves were filtered out of Gotu kola solution using a strainer. The Gotu kola solution was further diluted to 10, 15 and 20% v/v concentrations using sterile distilled water.

3.2.5 Potassium permanganate solution

Potassium permanganate powder was bought from a supermarket. It was then weighed out and dissolved in sterile distilled water to create 100, 150 and 200 ppm concentrations.

3. *E. coli* culture preparation

A colony of *E. coli* stock culture (Department of Industrial Microbiology, Suan Sunandha Rajabhat University) grown on Nutrient Agar (NA; Himedia) was transferred to 10 ml of Nutrient Broth (NB; Himedia). The culture was incubated at 35°C for 24 h. 3 ml of the grown culture was further transferred to 297 ml of NB in a 1,000-ml Erlenmeyer flask. The culture flask was incubated at 35°C for another 16 h and used to make an artificial contamination in lettuce leaf samples.

4. Lettuce washing experiments to investigate efficacies of five detergents in decontamination of *E. coli*

Lettuce samples were soaked in *E. coli* cell suspension for 2 min. The inoculated leaves were left to drain on a sterile metal screen in a biosafety cabinet for 30 min to allow cell attachment on the leaf surface. The contaminated leaves were then washed by dipping each single leaf separately in 200 ml of each cleaning solution (salt, vinegar, lime, Gotu kola and potassium permanganate solutions) as well as sterile tap water as a control. The leaves were soaked in the detergents for 10, 20 and 30 min. They were taken out of the tested solutions using sterile forceps and briefly immersed in sterile distilled water to rinse off the solutions. The washed leaves were put into stomacher bags containing 0.1% w/v peptone (Himedia) to help enrich the cell survivors and stomached for 1 min.

5. *E. coli* enumeration

The number of *E. coli* from pre-wash and post-wash was enumerated using traditional plating. The stomached lettuce samples were serially diluted using 0.1% peptone to obtain appropriate dilutions for culture quantification. 0.1 ml of the dilutions was then plated onto Eosin Methylene Blue agar (EMB; Merck) using spread plate technique. The plates were incubated at 35°C for 18-24 h. Colonies were calculated, and *E. coli* counts were reported in CFU/g and log CFU/g.

RESULTS AND DISCUSSIONS

Efficacies of four natural washing solutions including lime, vinegar, salt and Gotu kola solutions compared with potassium permanganate in destruction of *E. coli* artificially contaminated in lettuce were investigated in this study. Tap water, generally used for a routine produce wash, was incorporated in the tests for comparison. The infected lettuce was dipped in the tested detergents at various concentrations for 10, 20 and 30 min. The number of *E. coli* survivors at post-wash of each condition was calculated and displayed in Table 1.

The initial count of *E. coli* inoculated in lettuce at pre-wash was 9 x 10^7 CFU/g. After washing treatments with different cleaning solutions, lime solution was shown to be the most effective in disinfecting *E. coli* at tested conditions. The best condition resulting in the highest decline of *E. coli* cells was soaking in 15% lime solution for 30 min. This washing condition led to the least number of *E. coli* survivors (2.7 x 10^6 CFU/g) in the present study. The concentration of washing solution and the length of washing time were positively associated with the effectiveness of *E. coli* inactivation. To better contrast the killing effect of each detergent on *E. coli*, the decrease of *E. coli* cells following washing for 30 min was expressed in log CFU/g as shown in Table 2.

Table 1. Efficacy comparison of five cleaning solutions in decontamination of *E. coli* in lettuce in different washing conditions

Washing conditions	The number of *E. coli* survivors (CFU/g)		
	10 min	20 min	30 min
Pre-wash	9.12±0.02 x 10^7	9.12±0.02 x 10^7	9.12±0.02 x 10^7
Tap water	7.91±0.03 x 10^7	7.90±0.03 x 10^7	7.88±0.03 x 10^7
Lime solution 5%	6.68±0.03 x 10^7	4.63±0.04 x 10^7	2.48±0.07 x 10^7
Lime solution 10%	2.49±0.02 x 10^7	9.47±0.02 x 10^6	7.16±0.15 x 10^6
Lime solution 15%	6.71±0.02 x 10^6	4.63±0.02 x 10^6	2.69±0.15 x 10^6
$KMnO_4$ solution 100 ppm	7.65±0.02 x 10^7	6.60±0.02 x 10^7	5.53±0.03 x 10^7
$KMnO_4$ solution 150 ppm	5.53±0.09 x 10^7	4.50±0.09 x 10^7	3.45±0.05 x 10^7
$KMnO_4$ solution 200 ppm	3.71±0.02 x 10^7	2.63±0.02 x 10^7	1.69±0.05 x 10^7
Vinegar solution 5%	7.74±0.04 x 10^7	7.69±0.06 x 10^7	6.59±0.06 x 10^7
Vinegar solution 7.5%	5.89±0.03 x 10^7	4.76±0.02 x 10^7	3.91±0.02 x 10^7
Vinegar solution 10%	4.21±0.03 x 10^7	3.74±0.04 x 10^7	2.82±0.06 x 10^7
Gotu kola solution 10%	7.84±0.02 x 10^7	7.78±0.03 x 10^7	7.08±0.02 x 10^7
Gotu kola solution 15%	6.23±0.01 x 10^7	5.14±0.02 x 10^7	4.36±0.03 x 10^7
Gotu kola solution 20%	5.44±0.05 x 10^7	4.72±0.03 x 10^7	3.51±0.07 x 10^7
Salt solution 0.5%	7.85±0.11 x 10^7	7.80±0.07 x 10^7	7.71±0.08 x 10^7

Salt solution 1%	6.76±0.07 x 10^7	5.83±0.08 x 10^7	4.91±0.13 x 10^7
Salt solution 1.5%	5.88±0.04 x 10^7	5.10±0.10 x 10^7	4.08±0.02 x 10^7

Note: Data are presented in averages of three independent experiment ± standard deviations.

The most effective washing treatment in lowering *E. coli* contamination in lettuce at 30-min exposure time was 15% lime solution followed by 200 ppm potassium permanganate, 10% vinegar, 20% Gotu kola and 1.5% salt solutions, respectively. A 1.53-log reduction of *E. coli* was attained with 15% lime solution application whereas less than 1 log CFU/g was obtained with the other four cleaning solutions.

Lime juice brought about the highest antibacterial activity due to the composition of citric acid. When citric acid is present, it travels across the cell membrane and decreases the intracellular pH. The acidic environment inside the cell eventually causes cessation of enzymatic, protein, deoxyribonucleic acid (DNA) and nicotinamide adenine dinucleotide (NADH) functions leading to cell death [7]. In vinegar, acetic acid is the reason for its bactericidal activity. It has similar mechanisms of action to citric acid. The weak acid, acetic acid, migrates to the cytoplasm in an undissociated form. It then dissociates intracellularly liberating H^+ which is toxic to the cells. Gotu kola consists of triterpenoid compounds which exhibit the antibacterial activity [6]. Salt solution induces osmotic pressure in bacterial cells. It creates the concentration gradient between intracellular and extracellular liquid. Cells have to pump out cellular water to dilute the more concentrated liquid outside the cells. They are hence at risk of dying from cellular dehydration.

In current experiments, the bactericidal effect of these natural ingredients was not as outstanding. This could be owing to the low concentrations of the solutions used. Potassium permanganate solution was a little more powerful than vinegar, salt and Gotu kola solutions in killing *E. coli* in the tested conditions. However, all natural detergents studied were more effective than tap water in decontamination of *E. coli* in lettuce. Another explanation for the reduced efficacy of tested washing solutions was the high initial *E. coli* count of almost 8 log CFU/g at pre-wash. This *E. coli* quantity was a bit of an exaggeration from reality of actual contamination in the nature (<4 log CFU/g). The 30-min length of washing time could also be a little impractical in today's busy lifestyle. Accordingly, many areas can be improved for future studies to seek for best practices in vegetable washing. For instance, concentration of washing solution may be increased so as to decrease the exposure time. Mechanical force such as stirring and shaking may be added while washing to aid in a more thorough cleaning. The number of initial bacterial culture inoculated into the sample may be lessened to allow enhanced sanitizer efficacy.

Table 2. Efficacy comparison of five cleaning solutions in decontamination of *E. coli* in lettuce after soaking for 30 min

Washing solutions	*E. coli* survivors (CFU/g)	*E. coli* reduction (log CFU/g)
Pre-wash	9.12±0.02 x 10^7	-
Tap water	7.88±0.03 x 10^7	0.06
Lime solution 5%	2.48±0.07 x 10^7	0.57
Lime solution 10%	7.16±0.15 x 10^6	1.11
Lime solution 15%	2.69±0.15 x 10^6	1.53
$KMnO_4$ solution 100 ppm	5.53±0.03 x 10^7	0.22
$KMnO_4$ solution 150 ppm	3.45±0.05 x 10^7	0.42

$KMnO_4$ solution 200 ppm	$1.69 \pm 0.05 \times 10^7$	0.73
Vinegar solution 5%	$6.59 \pm 0.06 \times 10^7$	0.14
Vinegar solution 7.5%	$3.91 \pm 0.02 \times 10^7$	0.37
Vinegar solution 10%	$2.82 \pm 0.06 \times 10^7$	0.51
Gotu kola solution 10%	$7.08 \pm 0.02 \times 10^7$	0.11
Gotu kola solution 15%	$4.36 \pm 0.03 \times 10^7$	0.32
Gotu kola solution 20%	$3.51 \pm 0.07 \times 10^7$	0.42
Salt solution 0.5%	$7.71 \pm 0.08 \times 10^7$	0.07
Salt solution 1%	$4.91 \pm 0.13 \times 10^7$	0.27
Salt solution 1.5%	$4.08 \pm 0.02 \times 10^7$	0.35

Note: Data are presented in averages of three independent experiment ± standard deviations.

CONCLUSIONS

Among tested natural ingredients, 15% lime solution was the most effective in inactivating *E. coli* contaminated in lettuce achieving a 1.53-log reduction. The rest of the ingredients comprising 10% vinegar, 20% Gout kola and 1.5% salt solutions were not as potent as 15% lime solution and 200 ppm potassium permanganate in killing *E. coli* but better than tap water achieving less than 1 log CFU/g. The 30-min exposure to the detergents gave the greatest destruction of *E. coli*. Nevertheless, lowering initial cell count, applying mechanical force during washing, and utilizing more concentrated washing solutions were suggested for future directions to enhance the antibacterial activity of these natural sanitizers.

This study provided preliminary data for the development of suitable vegetable washing practice by making use of general household ingredients. As the healthy trend of eating fresh and less processed foods is on the rise, proper washing procedures prior to consumption are essential in reducing microbial contamination.

ACKNOWLEDGEMENTS

This work was funded by National Research Council of Thailand and Suan Sunandha Rajabhat University. The researchers would like to thank Institute of Research and Development, Suan Sunandha Rajabhat University in the support of research grant for this work. We would also like to thank the committees of the Personnel Development Fund of Suan Sunandha Rajabhat University for supporting registration fee and travelling expenses to join the International Academic Multidisciplines Research Conference (ICBTS) in Italy, March 2020.

REFERENCES

[1] National Bureau of Agricultural Commodity and Food Standards (ACFS). (2009). *Codex controls pathogens*. Retrieved December 29, 2019, from http://61.19.221.109/read_news.php?id=6582&ntype=09

[2] Numkham, L. & Purintharapibal. (2008). The situation of food sanitation at Thammasat University, Rangsit Campus. *Journal of Public Health*. 38, pp. 186-197.

[3] Lee, Y.U., Jo, S.H., Cho, S.D., Kim, G.H., Kim, Y.M. & Lee, D.H. (2009). Effects of chlorine concentrations and washing conditions on the reduction of microbiological contamination in lettuce. *Journal of the Korean Society for Applied Biological Chemistry*. 52, pp. 270-274.

[4] Singh, N., Singh, R.K., Bhunia, A.K. & Stroshine, R.L. (2002). Efficacy of chlorine dioxide, ozone, and thyme essential oil or a sequential washing in killing *Escherichia coli* O157:H7 on lettuce and baby carrots. *Lebensmittel-Wissenschaft und - Technologie*. 35, pp. 720-729.

[5] Panphut, W., Pudprommarat, C., Bulan, D. & Kumchoo, S. (2019). The study of sensorial evaluation screening for novel Thai curry paste. *Proceedings of the International Academic Multidisciplines Research Conference (ICBTS) in Amsterdam, the Netherlands on May 8-10, 2019*.

[6] Suwanpakdee, S. (2014). Efficiency of Gotu kola in *Escherichia coli* decontamination of ready-to-eat side vegetables. *Proceedings of the Ministry of Public Health Academic Conference in Nakornsrithammarat province, Thailand.* N.P.P.

[7] Su, L.-C., Xie, Z., Zhang, Y., Nguyen, K.T. & Yang, J. (2014). Study on the antimicrobial properties of citrate-based biodegradable polymers. *Frontiers in Bioengineering and Biotechnology*. 2, pp. 23-31.